INDIAN AGRICULTURAL RESEARCH INSTITUTE (PUSA)

IARI

जीव-जीवनेकृषि

LIBRARY

New Delhi

Call No.

Acc. No. 226275

Indian Agricultural Research Institute (Pusa)
LIBRARY, NEW DELHI-110012

This book can be issued on or after

Return Date	Return Date

3. Dog-earing of pages of a book, marking or writing therein with ink or pencil, tearing or taking out its pages or otherwise damaging it will constitute an injury to a book.
4. Unless a borrower points out the injury at the time of borrowing the book, he shall be required to replace the book or pay its price, if detected at the time of return.

HELP US TO KEEP THIS BOOK
FRESH AND CLEAN

SOIL SCIENCE

FOUNDED BY

RUTGERS COLLEGE

NEW BRUNSWICK, N. J.

JACOB G. LIPMAN, Editor-in-Chief

Assistant Editors

CARL R. WOODWARD

CHARLES A. DOEHLERT

IN CONSULTATION WITH

DR. F. J. ALWAY
University of Minnesota, St. Paul, Minn.

PROF. C. BARTHEL
Centralanstalten för Försöksväsendet på Jordbruksområdet, Stockholm, Sweden

DR. M. W. BEIJERINCK
Technische Hoogeschool, Delft, Holland

PROF. A. W. BLAIR
Rutgers College, New Brunswick, N. J.

DR. P. E. BROWN
Iowa State College of Agriculture, Ames, Iowa

ALBERT BRUNO
Ministry of Agriculture, Paris, France

DIRECTOR H. R. CHRISTENSEN
Statens Planteavls-Laboratorium, Copenhagen, Denmark

DR. H. J. CONN
New York State Experiment Station, Geneva, N. Y.

PROF. DR. H. VON FEILITZEN
Centralanstalten för Försöksväsendet på Jordbruksområdet, Stockholm, Sweden

DR. E. B. FRED
University of Wisconsin, Madison, Wis.

DR. J. E. GREAVES
Utah Agricultural College, Logan, Utah

DIRECTOR ACH. GRÉGOIRE
Agricultural Experiment Station, Gembloux, Belgium

DR. R. GREIG-SMITH
Linnean Society, Sydney, New South Wales

DR. B. L. HARTWELL
Rhode Island Experiment Station, Kingston, R. I.

DR. C. B. LIPMAN
University of California, Berkeley, Calif.

DR. BURTON E. LIVINGSTON
Johns Hopkins University, Baltimore, Md.

DR. F. LÖHNIS
U. S. Department of Agriculture, Washington, D. C.

DR. T. L. LYON
Cornell University, Ithaca, N. Y.

DR. M. M. MCCOOL
Michigan Agricultural College, East Lansing, Mich.

DR. W. H. MCINTIRE
Tennessee Experiment Station, Knoxville, Tenn.

DR. E. A. MITSCHERLICH
University of Königsberg, Prussia

PROF. C. A. MOOERS
Tennessee Experiment Station, Knoxville, Tenn.

DR. THEO. REMY
Institut für Boden- und Pflanzenbaulehre, Bonn a. Rh.

PROF. G. ROSSI
Royal Agricultural High School in Portici, Naples, Italy

DR. E. J. RUSSELL
Rothamsted Experimental Station, Harpenden, England

DR. O. SCHREINER
U. S. Department of Agriculture, Washington, D. C.

DR. ALEXIUS A. F. DE 'SIGMOND
Royal Joseph University of Technology, Budapest, Hungary

PROF. CHAS. E. THORNE
Ohio Experiment Station, Wooster, Ohio

PROF. N. M. TULAIKOV
Agricultural Experiment Station, Saratov, Russia

PUBLISHED MONTHLY BY

WILLIAMS & WILKINS COMPANY

BALTIMORE, MD., U. S. A.

SOIL SCIENCE

Contents for July, 1922

Neil E. Gordon and E. B. Starkey. Influence of Soil Colloids on Availability of Salts...... 1

A. W. Blair and A. L. Prince. Variation of Nitrate Nitrogen and pH Values of Soils from the Nitrogen Availability Plots........ 9

O. Arrhenius. A Possible Correlation Between the Fertility of Rice Soils and Their Titration Curves........ 21

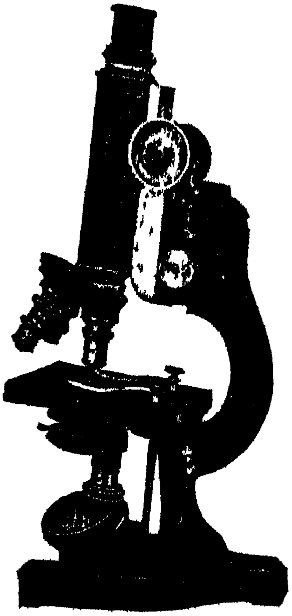
E. B. Fred and S. A. Waksman. Plate Method for Determining the Number of Microorganisms in the Soil........ 27

W. C. Frazier and E. B. Fred. Movement of Legume Bacteria in Soil........ 29

W. Rudolfs. Composting Rock Phosphate with Sulfur in Slightly Alkaline Calcareous Soils. . 37

Selman A. Waksman. The Influence of Soil Reaction upon the Growth of Actinomycetes Causing Potato Scab........ 61

Selman A. Waksman. Microbiological Analysis of Soil as an Index of Soil Fertility: I. The Mathematical Interpretation of Numbers of Microörganisms in the Soil........ 81



MICROSCOPES

AND MICROSCOPE ACCESSORIES

including microtomes, slides, covers and other accessories

We have all of the standard makes in stock

WRITE SPECIFYING YOUR REQUIREMENTS

EIMER & AMEND

Established 1851

HEADQUARTERS FOR LABORATORY APPARATUS AND CHEMICALS

NEW YORK, N. Y.

223 Third Ave.

SOIL SCIENCE

VOLUME XIV

JULY–DECEMBER, 1922

RUTGERS COLLEGE
NEW BRUNSWICK, NEW JERSEY
U. S. A.

PUBLISHED BY
WILLIAMS & WILKINS COMPANY
BALTIMORE, MARYLAND

SOIL SCIENCE

A MONTHLY JOURNAL DEVOTED TO PROBLEMS IN SOIL PHYSICS, SOIL CHEMISTRY AND SOIL BIOLOGY

JACOB G. LIPMAN, Editor-in-Chief

Assistant Editors
CARL R. WOODWARD
CHARLES A. DOEHLERT

IN CONSULTATION WITH

Dr. F. J. Alway, Univ. of Minnesota.
Prof. C. Barthel, Centralanstalten för Försöksväsendet pä Jordbruksomradet, Stockholm, Sweden.
Dr. M. W. Beijerinck, Technische-Hoogeschool, Delft, Holland.
Prof. A. W. Blair, Rutgers College.
Dr. P. E. Brown, Iowa State College of Agriculture.
Albert Bruno, Ministry of Agriculture, Paris, France.
Director H. R. Christensen, Statens Plantecavls-Laboratorium, Copenhagen, Denmark.
Dr. H. J. Conn, N. Y. State Exp. Station.
Prof. Dr. H. von Feilitzen, Centralanstalten för Försöksväsendet på Jordbruksomradet, Stockholm, Sweden.
Dr. E. B. Fred, University of Wisconsin.
Dr. J. E. Greaves, Utah Agricultural College.
Director Ach. Gregoire, Agricultural Experiment Station, Gembloux, Belgium.
Dr. R. Greig-Smith, Linnean Society, Sydney, New South Wales.
Dr. B. L. Hartwell, Rhode Island Experiment Station.
Dr. C. B. Lipman, Univ. of California.
Dr. Burton E. Livingston, Johns Hopkins University, Baltimore, Md.
Dr. F. Löhnis, U. S. Dept. of Agriculture
Dr. T. L. Lyon, Cornell University.
Dr. M. M. McCool, Michigan Agricultural College.
Dr. W. H. McIntire, Tenn. Exp. Station.
Dr. E. A. Mitscherlich, University of Königsberg, Prussia.
Prof. C. A. Mooers, Tenn. Exp. Station.
Dr. Theo. Remy, Institut für Boden- und Pflanzenbaulehre, Bonn a. Rh.
Prof. G. Rossi, Royal Agricultural High School in Portici, Naples, Italy.
Dr. E. J. Russell, Rothamsted Experiment Station, Harpenden, England.
Dr. O. Schreiner, U. S. Dept. of Agriculture.
Prof. Chas. E. Thorne, Ohio Experiment Station.
Prof. N. M. Tulaikov, Agricultural Experiment Station, Saratov, Russia.

Soil Science, founded in 1916 by Rutgers College, the New Jersey State College for the Benefit of Agriculture and the Mechanic Arts, is published monthly by the Williams & Wilkins Company, Baltimore, Md., U. S. A.

Entered as Second-Class Matter, August 23, 1918, at the Post Office at Baltimore, Maryland, under the Act of March 3, 1879.

Copyright 1922 by Williams & Wilkins Company

CONTENTS

Page

Influence of Soil Colloids on Availability of Salts. Neil E. Gordon and E. B. Starkey. 1

Variation of Nitrate Nitrogen and pH Values of Soils from the Nitrogen Availability Plots. A. W. Blair and A. L. Prince 9

A Possible Correlation Between the Fertility of Rice Soils and Their Titration Curves. O. Arrhenius 21

A Tentative Outline of the Plate Method for Determining the Number of Microorganisms in the Soil. S. A. Waksman and E. B. Fred 27

Movement of Legume Bacteria in Soil. W. C. Frazier and E. B. Fred 29

Composting Rock Phosphate with Sulfur in Slightly Alkaline Calcareous Soils. W. Rudolfs 37

The Influence of Soil Reaction upon the Growth of Actinomycetes Causing Potato Scab. S. A. Waksman 61

Microbiological Analysis of Soil as an Index of Soil Fertility: I. The Mathematical Interpretation of Numbers of Microörganisms in the Soil. S. A. Waksman 81

Differences Effected in the Protein Content of Grain by Applications of Nitrogen Made at Different Growing Periods of the Plants. W. F. Gericke 103

Some Relations of Arsenic to Plant Growth: Part 1. John Stewart 111

Some Relations of Arsenic to Plant Growth: Part 2. John Stewart and Edwin S. Smith 119

Nitrogen Fixation in Arid Climates. B. H. Wilsdon and Barkat Ali 127

Oxidation of Iron Pyrites by Sulfur-Oxidizing Organisms and Their Use for Making Mineral Phosphates Available. W. Rudolfs 135

A Microscopic Method for Demonstrating Fungi and Actinomycetes in Soil. H. J. Conn. 149

The Growth of Fungi in the Soil. Selman A. Waksman 153

Field Moisture Capacity and Wilting Point of Soils. W. L. Powers 159

Occurrence of Sulfides in Minnesota Peat Soils. Clayton O. Rost 167

The Human Element in the Work at Rothamsted 175

Variability of Alkali Soil. W. P. Kelley 177

The Soil Solution, Extracted by Lipman's Direct-Pressure Method, Compared with 1:5 Water Extracts. Paul S. Burgess 191

A Note on Oxidation of Sulfur in Oregon Soils. J. S. Joffe and H. C. McLean 217

The Potential Acidity of Soils. Olof Arrhenius 223

Effect of Certain Calcium Compounds and Other Substances on the Yield and Calcium Content of Some Crops. O. M. Shedd 233

Influence of Sulfur Oxidation upon Growth of Soy Beans and its Effect on Bacterial Flora of Soil. W. Rudolfs 247

Calcined Phosphatic Limestone as a Fertilizer. E. Van Alstine 265

Microbiological Analysis of Soil as an Index of Soil Fertility: II. Methods of the Study of Numbers of Microörganisms in the Soil. Selman A. Waksman 283

Nitrate Accumulation under Straw Mulch. W. A. Albrecht 299

Sulfur Oxidation in Inoculated and Uninoculated Greensand Mixtures and its Relation to the Availability of Potassium. W. Rudolfs 307

Microbiological Analysis of Soil as an Index of Soil Fertility: III. Influence of Fertilization upon Numbers of Microörganisms in the Soil. Selman A. Waksman 321

Silica and Silicates in Relation to Plant Growth and Composition. C. J. Schollenberger 347

The Sulfur Content of Rainwater. L. W. Erdman 363
Soil Experiment Fields and Their Value. Percy Edgar Brown 369
Some Ways of Increasing the Duty of Irrigation Water. W. L. Powers 377
A Study of the Causes of Frost Occurrence in Muck Soils. George Bouyoucos and M. M. McCool 383
The Relation of Soil Moisture to Physiological Salt Balance for Plants. John W. Shive. 391
The Effect of Nodule Bacteria on the Yield and Nitrogen Content of Canning Peas. E. B. Fred and O. C. Bryan 413
The Formation of Nodules by Different Varieties of Soybeans. E. B. Fred and O. C. Bryan 417
Sulfur as an Important Fertility Element. C. O. Swanson and W. L. Latshaw 421
Relation Between Heat of Wetting, Moisture Equivalent and Unfree Water. George J. Bouyoucos 431
Sulfur as a Fertilizer for Cotton. E. B. Reynolds and A. H. Leidigh 435
Adsorption of Plant Food by Colloidal Silica. R. C. Wiley and Neil E. Gordon 441
Influence of Hydrogen-Ion Concentration on the Adsorption of Plant Food by Soil Colloids. E. B. Starkey and Neil F. Gordon 449
Oxidation of Zinc Sulfide by Microörganisms. W. Rudolfs and André Helbronner 459
Modifications of the Soil Flora Induced by Applications of Crude Petroleum. I. L. Baldwin 465
Acid Phosphate Production by the Lipman Process: I. Effect of Moisture Content of Sulfur-Floats-Soil Mixtures on Sulfur Oxidation Activities. J. S. Joffe 479
A New Method of Mechanical Analysis of Soils. D. S. Jennings, M. D. Thomas and W. Gardner 485

ILLUSTRATIONS

PLATES

Variation of Nitrate Nitrogen and pH Values of Soils from the Nitrogen Availability Plots

Page
Plate 1. Fig. 1. The barley on plot 11A, without lime, was a failure. Fig. 2. Plot 11B with the same fertilizer treatment plus lime produced a good crop of barley....... 19

Movement of Legume Bacteria in Soil

Plate 1. Fig. 1. Second plant from inoculated end of box. Fig. 2. Third plant from inoculated end of box.......... 33
Plate 2. Fig. 3. Fourth plant from inoculated end of box. Fig. 4. Fifth plant from inoculated end of box.......... 35

Some Relations of Arsenic to Plant Growth: Part 2

Plate 1. Fig. 1. Wheat growing on soil treated with 0 to 500 parts of soluble arsenic per million parts of dry soil. Fig. 2. Beans growing on soil treated with 0 to 500 parts of soluble arsenic per million parts of dry soil.......... 123

Oxidation of Iron Pyrites by Sulfur-Oxidizing Organisms and Their Use for Making Mineral Phosphates Available

Plate 1. Plants grown in sand cultures with Shive's nutrient solution as basis and with additions of pyrites.......... 147

The Soil Solution Extracted by Lipman's Direct-Pressure Method, Compared with 1:5 Water Extracts

Plate 1. Fig. 1. Press No. 2, side view. Fig. 2. Press No. 2, end view.......... 213
Plate 2. Fig. 1. Press No. 3, shown in detail. Fig. 2. Press No. 3, assembled and resting on table of testing machine.......... 215

Influence of Sulfur Oxidation upon Growth of Soybeans and Its Effect on Bacterial Flora of Soil

Plate 1. Fig. 1. Soy bean plants grown in soil which had not received fertilizers for 30 years, sulfur alone added. Fig. 2. Soy bean plants grown in soil which had been fertilized for more than 30 years to which sulfur alone was added.......... 263

Calcined Phosphatic Limestone as a Fertilizer

Plate 1. Fig. 1. Phosphatic limestone in a phosphate rock mine near Columbia, Tennessee, after the overlying soil and the phosphate rock had been removed. Fig. 2. Sand cultures from series A. Fig. 3. Barley cultures in soil from series C—fertilizer salts (KN) and also the phosphates added to the soil for a previous crop of soy beans.......... 281

Sulfur Oxidation in Inoculated and Uninoculated Greensand Mixtures and Its Relation to the Availability of Potassium

Plate 1. Soy bean plants grown in quartz sand treated, etc.......... 319

Modifications of the Soil Flora Induced by Applications of Crude Petroleum

Plate 1. Petri plates inoculated with 1 cc. of a 1-100,000 dilution of soil.......... 477

TEXT FIGURES

Influence of Soil Colloids on Availability of Salts

Fig. 1. Relation of hydrogen-ion concentration to adsorption of potassium by silica gels. 4

A Possible Correlation Between the Fertility of Rice Soils and Their Titration Curves

Figs. 1, 2, and 3. Correlations between crop productivity and soil reaction........... 24

Composting Rock Phosphate with Sulfur in Slightly Alkaline Calcareous Soils

Fig. 1. Room temperature.. 41
2. 30° Centigrade.. 41
3. Apparatus used in aeration experiments.. 54

The Influence of Soil Reaction upon the Growth of Actinomycetes Causing Potato Scab

Fig. 1. Titration curves for the two soils.. 69

Nitrogen Fixation in Arid Climates

Fig. 1. Relation of rainfall and fluctuations in nitrogen content of Lyallpur soils...... 129

Field Moisture Capacity and Wilting Point of Soils

Fig. 1. Relation of soil class to field-moisture capacity.. 164

Variability of Alkali Soil

Fig. 1. Graphs showing the linear variation of an alkali soil, first foot, plot 3........... 181
2. Graphs showing the linear variation of an alkali soil, first foot, plot 8........... 183
3. Graphs showing the linear variation of an alkali soil at distances of two feet apart, first foot, series C.. 187
4. Graphs showing the linear variation of an alkali soil at distances of two feet apart, second foot, series C.. 188

Influence of Sulfur Oxidation upon Growth of Soy Beans and Its Effect on Bacterial Flora of Soil

Fig. 1. Graph showing the hydrogen-ion concentration at which rock phosphate becomes available.. 257
2. Curves showing changes of hydrogen-ion concentrations of soil 1 treated with different amounts of inoculated sulfur.. 259

Nitrate Accumulation under Straw Mulch

Fig. 1. Nitrate-nitrogen in soil under mulch as compared with unmulched soils........ 302
2. Nitrate-nitrogen in soil under mulch as compared with unmulched soil........ 302
3. Comparison of nitrate-nitrogen with percentage moisture in soil under mulch.. 303

Sulfur Oxidation in Inoculated and Uninoculated Greensand Mixtures and Its Relation to the Availability of Potassium

Fig. 1. Graphs showing the relation between hydrogen-ion concentration sulfates, acidity and water soluble potassium in compost mixtures................. 314

MICROBIOLOGICAL ANALYSIS OF SOIL AS AN INDEX OF SOIL FERTILITY: III. INFLUENCE OF FERTILIZATION UPON NUMBERS OF MICROÖRGANISMS IN THE SOIL

Fig. 1. Course of numbers of microörganisms in soil placed in container and kept in the laboratory at uniform moisture content. 323
2. Crop yields from plots and bacterial numbers. 337

SOME WAYS OF INCREASING THE DUTY OF IRRIGATION WATER

Fig. 1. Usable water capacity of soil classes. 378
2. Water requirement of crops on plots returning maximum net profit each year, based on a 12-year average. 380

THE RELATON OF SOIL MOISTURE TO PHYSIOLOGICAL SALT BALANCE FOR PLANTS

Fig. 1. Distribution of cultures producing the highest seven yields of buckwheat tops in each series of group I. 400
2. Average actual yields of buckwheat tops for low, medium and high soil-moisture of the cultures of group I. 401
3. Distribution of cultures producing the highest seven yields of buckwheat tops in each series of group II. 403
4. Average actual yields of buckwheat tops for low, medium and high soil-moisture of the cultures of group II. 405
5. Distribution of cultures producing the highest seven yields of buckwheat tops in each series of group III. 406
6. Average actual yields of buckwheat tops, for low, medium and high soil-moisture of the cultures of group III. 409

INFLUENCE OF HYDROGEN-ION CONCENTRATION ON THE ADSORPTION OF PLANT FOOD BY SOIL COLLOIDS

Fig. 1. Adsorption of K, NO_3, PO_4, and SO_4. 452

OXIDATION OF ZINC SULFIDE BY MICROÖRGANISMS

Fig. 1. Graph showing at which hydrogen-ion concentration the zinc of zinc sulfide becomes soluble. 463

MODIFICATIONS OF THE SOIL FLORA INDUCED BY APPLICATIONS OF CRUDE PETROLEUM

Fig. 1. Growth of dent corn in greenhouse, fifty-six days old. 473

A NEW METHOD OF MECHANICAL ANALYSIS OF SOILS

Fig. 1. Section of apparatus. 489
2 and 3. Distribution curves illustrating method of calculation. 489
4. Illustrative distribution curves (series 2) and the hypothetical curves (series 1) from which they were obtained. 491
5. Experimental data and distribution curves for two soils. 494
6. Distribution curves for two soils. 497

INFLUENCE OF SOIL COLLOIDS ON AVAILABILITY OF SALTS

NEIL E. GORDON AND E. B. STARKEY
University of Maryland

Received for publication October 13, 1921

INTRODUCTION

It has long been known that clays are hydrated mixtures of iron, aluminum, silica, the alkali metals and alkali earths, and that they also contain some organic matter. When the silicates decompose, the alkali and alkali earth metals become more or less available for plant-food while a part of the iron, alumina, silica, and organic matter (humus) take on the colloidal form, and in such form are not directly available as plant food. These colloids however, play an indirect and a very important rôle in plant nutrition as the experimental work, which is to follow, will show.

Many investigators have studied adsorption on clays where the clays have been treated as a whole, but in no case up to the present have they been able to definitely tell whether adsorption occurred or whether there was a chemical reaction between the clay and the salts used. Sullivan (4) showed that changes between radicals were involved. He found many complications especially when dealing with acid and alkaline salts. Van Bemmelen (5), Ashley (1) and Moore, et al. (3) of more recent date have also done work along this line.

For some time this laboratory has been trying to throw some light on the adsorption by colloids which are known to exist in the soils, and to find the relation between the hydrogen-ion concentration and adsorption as regards these colloids. It would seem that the work has shown how some salts in certain forms become available for plant-food.

MATERIAL USED

Hydrogels of silica, alumina, and iron were prepared in the purest possible condition. The salts used in the adorption work were potassium nitrate, potassium sulfate, potassium acid phosphate, calcium nitrate, calcium sulfate, calcium acid phosphate, magnesium nitrate, magnesium sulfate, and magnesium acid phosphate. The salts were those put out by the J. T. Baker Chemical Company, under the direction of the National Research Council.

EXPERIMENTAL RESULTS

Weighed quantities of the gels were placed in glass stoppered bottles with the various solutions and shaken until equilibrium was established. The time required for the equilibrium depended upon the amount of shaking and upon the kind of gel and salt used. The solutions were analyzed before and after shaking and the adsorption obtained by difference. All the water of hydration was considered water of dilution in order that the minimum adsorption might be shown. The adsorption of each ion was calculated. In the case of calcium acid phosphate, adsorptions were obtained as shown in table 1.

In case of silica there is slight adsorption or negative adsorption. The negative adsorption would indicate that all the water which is in the gel does not act as water of dilution. Both ions are very highly adsorbed by the alumina and iron gels, and the adsorption increases with an increase of concentration.

When the silica, alumina, and iron gels had adsorbed their maximum quantity of calcium acid phosphate they were washed with hot water. The first 50 cc. of wash water was analyzed except in the case of alumina. Then 500 cc. was passed through in about 50-cc. portions and another 50-cc. portion was analyzed. This was continued during the day, and then a 50-cc. portion was allowed to stand on the gels over night. This 50 cc. was run off in the morning and analyzed in order to compare this portion with the portion obtained the day before. Table 2 gives the results for the phosphate ion.

TABLE 1

Adsorption of calcium acid phosphate by silica, alumina, and iron gels

CONCENTRATION	Ca ADSORBED PER GRAM GEL			PO_4 ADSORBED PER GRAM GEL		
	Silica	Alumina	Iron	Silica	Alumina	Iron
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
0.100 *N*................	−0.12	84.9	121.4	0.08	610.2	609.9
0.050 *N*................	−0.12	51.4	83.3	0.04	393.4	421.6
0.025 *N*................	−0.11	32.7	54.5	0.01	272.6	266.6

From table 2 it is quite plain in what order the adsorbed salts are washed from the gel. The first washing gives a heavy yield of phosphate, as it takes out a large part of the salt that is mechanically held. The amount in the filtrate slowly and gradually decreases as long as there is continuous washing, but when water is allowed to stand on the gel over night, the drop is much less marked in the case of iron and alumina gels, and in the case of silica there is a noticeable increase. It took about 20 liters of washings before the phosphate was washed from the silica so that it would not give a test when water was allowed to stand on it over night. The point was not reached when the wash water of the alumina and iron gel was entirely free from phosphate.

The silica gel was washed until it did not give a trace in its washings and then the gel was decomposed and only a trace of phosphate was found. When the washings from the alumina and iron gels began to give the small values (0.3 mgm.) given in table 2, they were decomposed and the phosphate left in the gels determined. These results are given in table 3.

TABLE 2

Results obtained by analyzing a series of washings from silica, alumina and iron gels after each had suffered a maximum adsorption of $Ca(H_2PO_4)_2$

TIME OF ANALYSIS	PO_4 FOUND IN 50 CC. OF WASH WATER		
	Silica gel	Alumina gel	Iron gel
	mgm.	*mgm.*	*mgm.*
First 50 cc.	72.9		39.9
After 500 cc.	0.5	4.0	7.8
After 1000 cc.	0.4	2.6	3.7
After 1500 cc.	0.3	2.3	2.4
After first night	5.9	2.6	2.1
After 3000 cc.	0.0	2.0	1.8
After second night	4.0	1.8	1.8
After 5000 cc.	0.0	0.3	0.2
After third night	Trace	0.3	0.3

TABLE 3

Results obtained by analyzing alumina and iron gels for PO_4 radical before and after washings

TIME OF ANALYSIS	PO_4 IN GEL	
	Alumina gel	Iron gel
	gm.	*gm.*
Before washing	0.3142	0.5728
After washing	0.2308	0.2688

TABLE 4

Results which show how adsorption varies with the hydrogen-ion concentration

pH VALUE	ADSORPTION PER GRAM SILICA GEL		pH VALUE	ADSORPTION PER GRAM IRON GEL	
	Ca	PO_4		Ca	PO_4
	mgm.	*mgm.*		*mgm.*	*mgm.*
4.243	0.45	−0.64	5.156	109.5	554.8
4.193	0.41	−0.75	5.156	104.5	536.8
4.193	0.36	−1.00	5.038	101.9	536.8
4.142	0.18	−1.56	5.021	101.9	581.7
2.463	−0.89	−2.62	4.446	56.4	587.7

The alumina gel contained about two-thirds of the original phosphate radical, while the iron gel contained about one-half.

One-twentieth normal solutions were made of different salts and their hydrogen-ion concentration was varied by the addition of sodium hydroxide or hydrochloric acid as the case might require. The adsorption in the case of calcium acid phosphate is given in table 4.

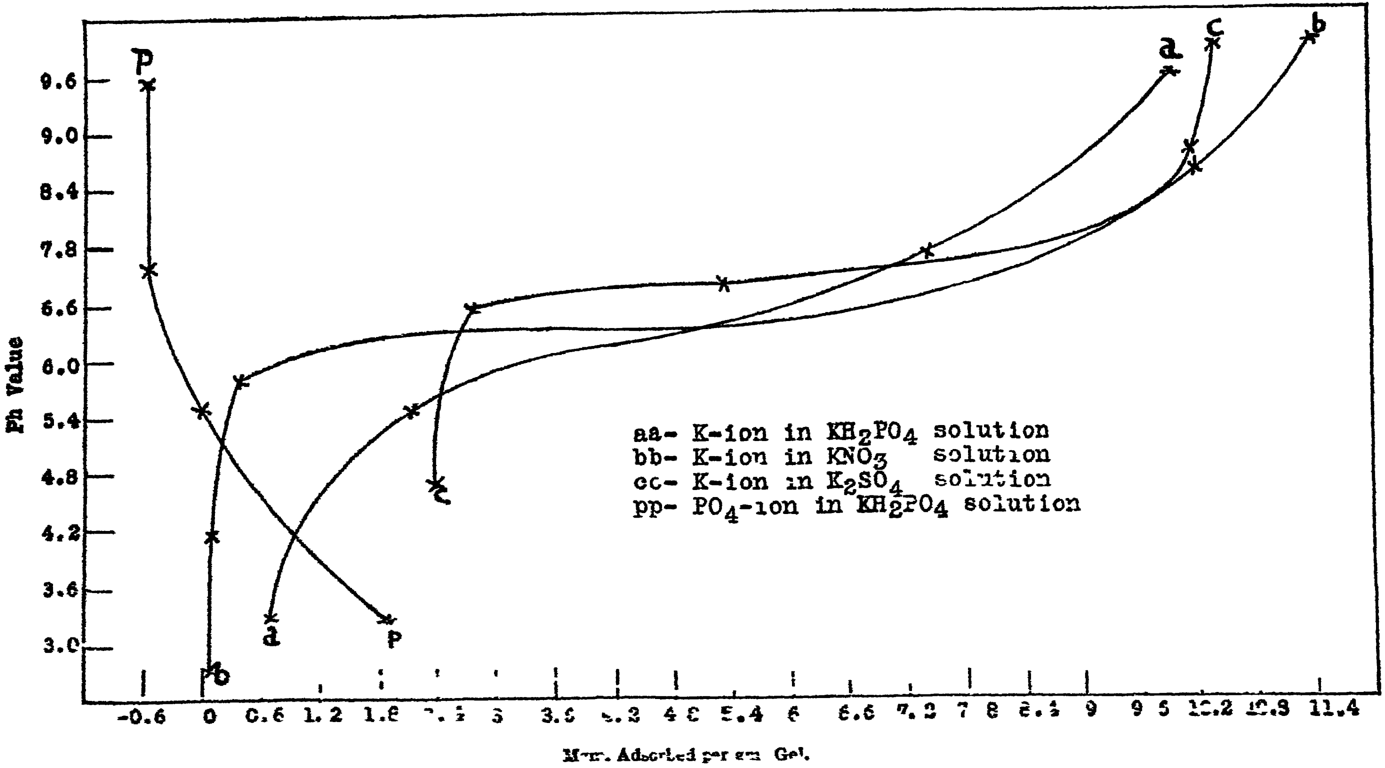


FIG. 1. RELATION OF HYDROGEN-ION CONCENTRATION TO ADSORPTION OF POTASSIUM BY SILICA GELS

Table 4 shows how the amount of calcium adsorbed in each gel decreases with an increase of hydrogen-ion concentration. The phosphate radical follows the same order in the silica gel, but in the iron gel the order seems to be somewhat broken. The change of adsorption is not so marked in any case because of the small change in hydrogen-ion concentration. The hydrogen-ion concentration had to be kept within such limits that the calcium acid phosphate would not be precipitated on the one hand and that the iron would not dissolve on the other hand.

How the adsorption of potassium varies with a change of hydrogen-ion concentration when silica gel is treated with various potassium salts, is shown in table 5.

Potassium in potassium salts follow the same order as calcium in calcium salts but table 5 shows that in the case of silica gel potassium is much more adsorbed, and further shows a greater change of adsorption with a change of hyrogen-ion concentration. A graph, figure 1, of the results of table 5 brings out the relation between hydrogen-ion concentration and adsorption. It is noted by the graph that when the hydrogen-ion concentration is about 7, a small change in hydrogen-ion concentration is accompanied by a large change in adsorption.

TABLE 5

Variation of adsorption of potassium in relation to hydrogen-ion concentration when solutions of different potassium salts are used with silica gel

ADSORPTION OF KH_2PO_4		ADSORPTION OF KNO_3		ADSORPTION OF K_2SO_4	
pH value	Amount per gram of gel	pH value	Amount per gram of gel	pH value	Amount per gram of gel
	mgm.		*mgm.*		*mgm.*
9.501	9.63	9.873	11.2	9.890	10.06
7.692	6.56	8.554	9.8	8.706	9.93
6.086	1.74	6.830	2.6	7.337	4.70
3.888	−0.68	6.390	−1.1	7.117	1.91
		3.360	−1.3	5.275	1 16

DISCUSSION OF RESULTS

The results in table 1 would indicate that the adsorption of soils is influenced more by iron and alumina colloids than by silica colloids. The alumina and iron colloids would have about equal influence. The concentration of the soil solutions should have some influence on the quantity of salt adsorbed by the soil colloids.

Table 2 gives one some idea of how salts may become available after they have once been adsorbed by the colloids of the soil. The adsorbed quantity, which would probably be the amount left after the second or third washing is seen to be released in a very gradual fashion. This indicates that soil colloids play a rôle in keeping salts from being washed quickly from the soil. When the water is allowed to stand on the gel over night, the salt is given time to partition itself to a greater extent between the gel and the water. In the adsorption experiments it was found that the gels reached an equilibrium very slowly, and in a like manner they give back their salts very slowly, but probably fast enough for the use of the salt for the plant. This fact is especially true of the iron and alumina gels. Whether all the salt adsorbed by the alumina and iron gels is available for plant food is questionable.

The results as given in table 4 and the accompanying graph point to the fact that the adsorption by soil colloids is influenced to some extent, at least, by the acidity of the soils. Potassium and calcium in certain forms, at least, are less available as the hyrogen-ion decreases. In the graph, figure 1, it will be noted that a small change in the hydrogen-ion concentration around the value 7 is followed by a big change in adsorption of potassium by silica gel.

The work seems to be in keeping with some investigational work on soils. The work of A. G. McCall and A. M. Smith (2) is an illustration. They added sulfur to different greensand marls, and varied conditions so that varying amounts of sulfur were oxidized over to the sulfur acids. Where the greatest amount of sulfur was oxidized the largest quantity of water soluble potassium was produced. According to table 4, when the hydrogen-ion concentration is increased by the oxidation of the sulfur, the potassium should become less adsorbed or more available. This is what was found in the reference given.

The probable facts are that the outer surfaces of these silicate grains have undergone decomposition to the extent that the iron, alumina, and silica in this outer layer have taken on the colloidal form. Each sand grain has become, as it were, encased in a thin colloidal layer and the potassium is more or less adsorbed by this colloid. As soon as the sulfur begins to be oxidized, and hence the hydrogen-ion concentration increased, the potassium, according to table 4, should be less adsorbed by this encasing colloid or become more available. This is what McCall and Smith found. Furthermore, they found that the fineness of the sand increased the availability of the potassium. Since the fineness of the sand means more surface, and since adsorption is a surface phenomena this increased availability of the potassium would be expected when the hydrogen-ion concentration is increased by the addition of sulfur.

SUMMARY

1. Silica gel shows small adsorption for salts like calcium acid phosphate, while the alumina and iron gels show a large power of adsorption.

2. Within the limits of the concentrations used, the adsorption increased with the concentration of the salt solution.

3. The colloids gave up their adsorbed salts gradually.

4. One of the factors affecting the solubility and therefore the availability of some ions like potassium and calcium in certain forms is the hydrogen-ion concentration.

REFERENCES

(1) Ashley, H. E. 1915 Technical control of the colloidal matter of clays. U. S. Bur. Stan. Technol., Paper 23.

(2) McCall, A. G., and Smith, A. M. 1920 Effect of manure-sulfur composts upon the availability of the potassium of greensand. *In* Jour. Agr. Res., v. 19, p. 239–256.

(3) Moore, C. J., W. H. Fry, Middleton, H. E. 1921 Methods for determining the amount of colloidal material in soils. *In* Jour. Indus. Engin. Chem., v. *13*, p. 527–530.

(4) Sullivan, E. C. 1907 The interaction between mineral and water solutions with special reference to geologic phenomena. U. S. Geol. Sur. Bull. 312, v. 69, p. 5–64.

(5) Van Bemmelen, J. M. 1879 Das Absorptionvermögen der Ackererde. *In* Landw. Vers. Stat., v. 23, p. 265–393.

VARIATION OF NITRATE NITROGEN AND pH VALUES OF SOILS FROM THE NITROGEN AVAILABILITY PLOTS[1]

A. W. BLAIR AND A. L. PRINCE

New Jersey Agricultural Experiment Stations

Received for publication November 21, 1921

The nitrogen availability plots at this station have been under observation since 1908 and for this reason they seemed to offer an excellent opportunity for a study of the variations in pH values and nitrate nitrogen in the soil during and immediately following the growing season.

It was therefore determined to take samples at intervals of about two weeks through the summer and continuing into the fall as long as samples could be taken to good advantage.

The crop rotation which has been used and the fertilizer treatment have been described in earlier publications (2, 3) and on that account only brief reference need be made here to these points. The soil is a loam, but inclines to the gravelly phase.

The rotation is a general farm crops rotation—corn, oats, wheat and timothy (two years) without legumes, since these could not be introduced where the availability of nitrogenous fertilizers is being studied. The series of plots marked *A* receive no lime; those marked *B* received ground limestone at the rate of one ton per acre in 1908 when the work was started, and since that time, at intervals of five years, have received two tons per acre. The last application was made in the spring of 1918.

The fertilizer treatment for the plots included has been as follows:

PLOT NUMBER		FERTILIZER TREATMENT
7A,	7B	Nothing
9A,	9B	Minerals,[1] 16 pounds $NaNO_3$
11A,	11B	Minerals, $(NH_4)_2SO_4$ equivalent to 16 pounds $NaNO_3$
13A,	13B	Minerals, dried blood equivalent to 16 pounds $NaNO_3$
17A,	17B	Minerals, 200 pounds wheat or rye straw
18A,	18B	Minerals, 1600 pounds cow manure and 16 pounds $NaNO_3$
19A,	19B	Minerals, only

[1] Minerals = 32 pounds acid phosphate and 16 pounds muriate of potash.

Fertilizers and manures are applied annually, near the time of seeding the crop, except in the case of fall-sown crops, when only one-fourth of the mineral nitrogenous fertilizer is applied at the time of seeding and the remainder as a top dressing the following spring. Also in the case of the hay crop the mineral fertilizers are applied in the fall following the first season's cutting.

---

[1] Paper No. 96 of the Journal Series, New Jersey Agricultural Experiment Stations, Department of Soil Chemistry and Bacteriology.

This paper will appear in the Rutgers College Studies, vol. 1.

It should be explained here that the wheat which was seeded in the fall of 1919 was badly injured by the cold weather of 1919-1920 and on this account it became necessary to plow this under and reseed to barley in the spring of 1920. The barley was seeded on May 3. Nearly a month later—May 29—the first samples of soil were collected. Five or more borings, to the depth of 6⅔ inches, were taken from each plot, and these were combined to make a composite sample. These samples were taken to the laboratory where they were air-dried and passed through a 2 mm. sieve (without grinding) to remove gravel and course organic matter. Following the barley the land was plowed during the latter half of August and fitted for seeding timothy. Manure and fertilizers were applied in accordance with the plan already outlined, the former being spread broadcast before the land was plowed and the latter after the land was prepared, September 3 and 4. The timothy was seeded September 7.

The hydrogen-ion determinations were made by the double tube color method described by Gillespie (1) and the nitrate determinations by the colorimetric method of Schreiner and Failyer (5).

The results of the hydrogen-ion determinations (pH values) are shown in table 1. It is at once apparent that there has been little change in the pH values throughout the period, the averages for the various samplings being almost identical, with the exception of the samples taken May 29th.

There is on the other hand a distinct difference between samples from the limed and the unlimed plots, the latter showing distinctly lower pH values, the lowest season-average for any plot being 4.7 (11A), and the highest 6.1 (9A and 18A).

It will be recalled that 11A is the plot which has received annual applications of ammonium sulfate equivalent to 320 pounds of nitrate of soda per acre. This plot is now so acid that it practically inhibits growth of such crops as corn, wheat, and timothy. Instead there appears a volunteer crop of sour grass, and later in the season a heavy growth of crab grass.

From the results of this work it would appear that a pH value in the neighborhood of 4.5 indicates a degree of acidity which practically prohibits the growth of the ordinary farm crops. This view is strengthened by results secured where the soil was made very acid by heavy applications of sulfur (4). In this case the growth of soy beans was very much reduced where the pH value was 4.5 or less.

With the exception of 11B (the limed ammonium sulfate plot) the limed plots show a pH value varying but little from 7.0. However the samples taken on May 29th and November 25th appear to be slightly lower in pH value than those taken at the intervening dates.

The samples from 11B are consistently lower in pH value than those from the other plots, although the soil of this plot is not sufficiently acid to interfere with normal crop growth.

It is of interest to compare pH values of samples from these plots with the lime requirement determination by the Veitch method. These determinations were made for all of the plots, but at less frequent intervals than the pH readings. It does not seem wise to attempt a direct comparison with the pH values, but it is of interest in connection with these values to note that the average lime requirement for plot 11A is more than four times as much as for 9A.

TABLE 1

Hydrogen-ion concentration, expressed as pH values, of soils from the nitrogen availability fertilizer plots. May 29 to November 25, 1920

PLOT NUMBER	MAY 29	JUNE 14	JUNE 28	JULY 12	JULY 26	AUGUST 9	AUGUST 24	SEPTEMBER 13	SEPTEMBER 27	OCTOBER 11	OCTOBER 25	NOVEMBER 11	NOVEMBER 25	AVERAGE
Unlimed section														
	pH	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*
7A*	5.8	5.8	6.0	5.8	6.0	6.0	6.0	5.8	6.0	6.2	6.2	6.1	6.0	5.97
9A	6.0	6.4	6.3	6.1	6.0	6.0	5.9	6.0	6.0	6.2	6.1	6.0	6.0	6.07
11A	4.6	4.5	4.6	4.7	4.6	4.8	4.9	4.9	4.5	4.7	4.9	4.7	4.7	4.70
13A	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.9	5.9	5.9	5.8	6.0	5.9	5.79
17A	5.8	6.0	6.2	6.0	5.8	5.8	5.8	5.8	5.9	5.8	5.8	5.9	6.0	5.89
18A	5.9	6.2	6.2	6.2	6.0	6.1	6.3	6.2	5.9	6.0	6.0	6.0	6.0	6.07
19A	5.7	5.4	5.4	5.9	5.7	5.7	5.9	6.0	5.7	5.8	5.9	5.8	5.9	5.75
Average.........	5.61	5.70	5.73	5.76	5.63	5.70	5.75	5.80	5.65	5.73	5.75	5.73	5.70	5.71
Limed section														
7B	6.9	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.1	7.1	7.0	7.0	7.0	7.01
9B	7.1	7.2	7.1	7.2	7.1	7.2	7.2	7.1	7.2	7.0	7.0	7.1	7.1	7.02
11B	6.4	6.5	6.4	6.8	6.5	6.7	6.7	6.8	6.6	6.6	6.6	6.4	6.4	6.56
13B	6.9	6.9	6.9	6.9	7.0	7.0	7.1	7.1	7.1	7.0	7.0	6.8	6.9	6.96
17B	6.9	7.2	7.1	7.1	7.1	7.1	7.1	7.1	7.2	7.2	7.0	6.9	6.8	7.06
18B	6.9	7.1	7.1	7.1	7.1	7.1	7.1	7.2	7.2	7.2	7.1	7.0	6.9	7.08
19B	7.1	7.1	7.1	7.1	7.2	7.2	7.2	7.2	7.2	7.2	7.1	7.1	6.9	7.13
Average.........	6.89	7.00	6.96	7.03	7.00	7.04	7.06	7.07	7.08	7.04	6.97	6.90	6.86	6.99

*Omitted from average on account of the difficulty in getting a clear solution for readings.

The lime requirement appears to vary some with the date of sampling, but this variation may be apparent rather than real, since it is difficult to get close agreement of results by this method.

Of the limed plots, 9A shows a lower average requirement than any of those that received fertilizer. This is in comformity with the generally accepted opinion that the continuous use of nitrate of soda tends toward an alkaline condition of the soil. In this connection it may be pointed out that 9A shows a higher pH value than any of the other unlimed plots except 18A, and this plot also receives nitrate of soda in addition to the manure.

Of the limed plots only 11B showed a lime requirement by the Veitch method, and this is so low as to be practically negligible. The lime requirement determinations are shown in table 2.

TABLE 2

Lime requirement (pounds CaO per 2,000,000 pounds soil) by the Veitch method. 1920

DATE OF SAMPLING	7A	9A	11A	13A	17A	18A	19A	AVERAGE
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
May 29	400	600	3200	1400	1200	800	1200	1257
July 12	600	600	2000	800	800	800	1200	971
August 24	400	600	1600	1200	400	800	1200	886
October 11	200	200	2000	600	1000	1000	1200	886
November 25	600	600	1800	600	1000	1000	800	771
Average	360	520	2120	920	760	880	1120	954

INFLUENCE OF SOIL REACTION ON CROP YIELDS

In connection with the pH values and lime requirement data it is of interest to study the yields of barley and of timothy hay obtained from these plots for the seasons of 1920 and 1921. The weights for the different plots are shown in table 3 which appears under the discussion of nitrates. From these figures it will be noted that the limed plots gave the largest yield in nearly every case. The low yields from 7A and 7B are due in part to starvation, these plots having received no fertilizer during the entire period. The wide difference between the yields on 11A and 11B is of especial interest in connection with the pH values and lime requirement determinations for these plots, the yield of barley on the latter being eight times, and of timothy hay nearly twenty-five times that on the former. In connection with the timothy hay of 1921 it should be pointed out, that 11A yielded a rather heavy second growth crop, 660 pounds, which was practically all crab grass. On the other hand there was not enough second crop growth on 11B to justify cutting.

The increase in yield from 17B over 17A is due in part to volunteer clover which has been gradually creeping in on this plot. The tendency of the clover to come in is due to the fact that this plot receives its nitrogen in the form of chopped-up rye straw, a slowly available source, and the lime and minerals which are applied encourage the growth of clover to the exclusion of the timothy which does not get enough available nitrogen. A considerable amount of volunteer clover has also appeared on 19B and this accounts in part for the increase in yield over 19A. These two cases only serve to further emphasize the importance of keeping the soil reaction near the neutral point where clover is to be grown.

NITRATE NITROGEN IN THE SOILS

Nitrate determinations for the seven limed and seven unlimed plots, for the period May 29 to November 25, 1920, are shown in table 3.

With comparatively few exceptions the unlimed plots show a higher nitrate content than the limed plots. This is clearly brought out by the averages for the two sections, the general average for the former being 4.30 parts of nitrogen per million and for the latter 3.42 parts per million.

TABLE 3

Nitrate nitrogen expressed as parts per million of nitrogen in soil samples from unlimed and limed plots collected at intervals of two weeks from May 29 to November 25, 1920

PLOT NUMBER	MAY 29	JUNE 14	JUNE 28	JULY 12	JULY 26	AUGUST 9	AUGUST 24	SEPTEMBER 13	SEPTEMBER 27	OCTOBER 11	OCTOBER 25	NOVEMBER 8	NOVEMBER 25	AVERAGE
Unlimed section														
	p.p.m	*p.p m.*	*p.p.m.*	*p.p m.*	*p.p.m*	*p.p.m*	*p.p.m*	*p.p m*	*p.p m*	*p p.m*	*p p m*	*p.p.m*	*p.p.m.*	*p.p.m.*
7A	2.52	1.26	4.12	2.12	2.10	0.72	1.46	5.13	5.73	4.00	8.79	8.79	1.10	3.68
9A	5.65	0.88	2.14	1.61	1.20	1 08	4.00	6.91	11.46	6 87	4.24	1.80	0.80	3.74
11A	8.27	13.18	20.78	16.46	0 66	0 50	2.26	4.00	7.99	3.49	7.99	7.99	1.72	7.33
13A	14.39	1 76	1.44	2.00	0 80	0 72	2.52	3.32	7.03	4 00	2.50	1.53	1.34	3.33
17A	2.60	0.72	1.30	1.06	0 80	0.61	1.04	1.70	2 40	2.66	3 66	2.28	1.00	1.67
18A	13.79	2.80	4.19	3.46	3.06	4.60	2 92	15.98	21.98	9.99	14.59	13.26	1.80	8.63
19A	3.25	1.16	1.34	1.90	0.80	0 53	0.81	2.00	2.54	2.00	2.20	3.04	0.64	1.71
Average	7.21	3.11	5.04	4.09	1.35	1.25	2.14	5.58	8.45	4.72	6.28	5.53	1.20	4.30
Limed section														
7B	3.86	1.26	1.40	2.00	1.60	0.69	1.33	6.63	13.83	2.00	4.00	4.63	0.80	3.39
9B	5.32	0.46	1.60	1.75	3.20	1.00	1.90	7.43	7.99	2.00	3.06	1.06	0.72	2.88
11B	7.99	1.00	1.54	2.00	2.00	1.36	1.04	4 80	4.00	3.66	3.20	3.60	0.80	2.85
13B	5.51	0.92	1.50	2.12	2.00	0.90	1.72	3.52	5.27	3.66	5.00	6.39	1.60	3.09
17B	5.79	3.20	2.60	4.00	3.00	1.44	1.90	4.63	6.63	2.00	1.80	1.80	1.00	3.06
18B	9.31	0.88	3.20	4.00	3.46	3.32	4.52	15.98	22.37	6.33	1.91	2.00	1.60	6.07
19B	3.39	0.94	2.14	2.04	2.26	1.76	2.32	5.31	7.19	1.28	2.60	0.88	1.60	2.59
Average	5.88	1.24	2.00	2.56	2.50	1.50	2.10	6.90	9.61	2.99	3.08	2.91	1.16	3.42

The larger growth of barley on the limed plots during the early part of the season might easily account for this difference. On the other hand there is some doubt as to whether this explanation would hold after the barley was full ripe and had been harvested, that is, after about July 26.

During the early part of the season the barley made very little growth on 11A and this was reflected in the higher content of nitrates in the soil up to July 12. The low nitrate content of this soil for July 26 and August 9 appears to be accompanied by a low content on nearly all the other plots. Since the grain was full ripe on July 26 and was harvested between that date and the end of the month, it could hardly be attributed to the withdrawal of nitrogen by the growing crop.

The rainfall during the months of July and August was heavy—over 14 inches for the two months—and it is entirely possible that this may have kept the nitrates washed below the depth to which soil samples were taken. During September and October when the amount of nitrate nitrogen was greater, the rainfall averaged only a little over two inches for the two months. It must not be forgotten, however, that the application of the fertilizer early in September had something to do with the higher nitrate content at this time. Likewise the high average content of nitrates for May 29 is undoubtedly a reflection of the nitrogenous fertilizers which were applied to certain of these plots (no. 9, 11, 13, 17 and 18) on April 9.

The average nitrate content of the soil from plot 11A was 7.33 parts per million which is next to the highest average, the highest being 8.63 from plot 18A, which receives annually a heavy application of manure in addition to the nitrate of soda.

From November 8 to 25 there was a rather abrupt drop in the amount of nitrates for nearly all plots. As the first freezing weather occurred about November 12 to 14, the lower temperatures from this time on would no doubt account for the slowing down of nitrification.

The low average for 17A may be attributed to the fact that this plot receives rye straw which furnishes a comparatively small amount of nitrogen in a slowly available form. Plot 19A receives no nitrogen and here also the average is low.

There is less variation in the amount of nitrate nitrogen in the soils from the limed than from the unlimed section, the average being not quite three parts per million if 18B be excepted.

The use of lime and mineral fertilizers on 17B and 19B has encouraged the growth of volunteer clover on these plots, and this undoubtedly accounts, in part, for the higher production of nitrates on these plots than on the corresponding unlimed plots.

The highest average nitrate content for both sections occurred on September 27 when the plots of the unlimed section gave an average of 8.45 parts of nitrogen per million and the plots of the limed section 9.61 parts per million. Two weeks later the average of the plots for the limed section was less than one-third what it was on September 27. The reason for the high content of nitrates for the two samplings in September has already been referred to.

NITRATE FORMATION AND CROP YIELD

As already pointed out plot 11A shows a higher season average of nitrates found in the soil than 11B, and yet the yield of barley (grain) on the former for 1920 was approximately $\frac{1}{25}$, and the timothy hay in 1921, only $\frac{1}{3}$ of that on the latter.

It is thus quite clear that growth was hindered by something else than a lack of available nitrogen, and that an acid soil does not necessarily inhibit nitrification. In an earlier publication (4) attention has been called to this latter fact.

The yields for the two crops are shown in table 4.

It will be noted that the yield of timothy hay on plots 9A and 9B, 13A and 13B, and 18A and 18B do not differ greatly, although 18A and 18B receive about four times as much nitrogen every year as the others. From this it is evident that there must be a very great loss of nitrogen from these two plots.

This excessive application of nitrogen tends to produce a hay or grain crop with a weak straw and in the case of grain crops, an over abundance of leaf and stem growth as compared with grain and consequently there is a considerable loss on account of lodging.

TABLE 4

Yield of barley (grain) and timothy hay on unlimed and limed plots 1920 and 1921

PLOT NUMBER	BARLEY, 1920		TIMOTHY HAY, 1921	
	A (Unlimed)	B (Limed)	A (Unlimed)	B (Limed)
	lbs.	*lbs.*	*lbs*	*lbs.*
7	81	568	151	650
9	1308	1244	2880	2960
11	56	1360	450	3620
13	1112	1240	2700	3000
17	864	1340	1580	2120
18	1888	2040	2760	3440
19	604	960	870	1650

During the spring and early summer of 1921 there was a very heavy growth of chick weed on plots 18A and 18B, and this interfered very seriously with the growth of the timothy. It would appear that the abundant supply of nitrates is especially favorable to the growth of this weed. It is thus evident that an over-supply of nitrates may have a distinctly detrimental effect.

The low yields on plots 7A and 7B furnish a good illustration of how the yields go down on land that is continuously unmanured and unfertilized. It would appear that the lime continues to aid in unlocking slowly available nitrogen and mineral plant food and thus the yield on the limed plot is kept considerably above that on the corresponding unlimed plot. However, the average amount of nitrates is very nearly the same on the two plots.

It is only a matter of time when even the limed plot will fail to give a crop worth harvesting if the same treatment is continued.

In the case of 17B the lime has apparently aided in breaking down the slowly available organic matter and this has resulted in a larger supply of nitrates for the crop, but as previously pointed out this effect may be apparent rather than real, and the larger crop yield and higher nitrate concentration may be due to the volunteer clover which has been creeping in from year to year. On account of a deficiency of lime there is no tendency for volunteer clover to appear on the corresponding unlimed plot, 17A.

SUMMARY

Hydrogen-ion determinations on sampes of soil collected at intervals of two weeks, over a period of nearly 5 months, showed only slight variations in pH values for a given fertilizer treatment.

On the unlimed section the pH values varied considerably according to the fertilizer treatment given the different plots; the variation was much less, for the same treatment, on the limed section. The pH values were quite consistently lower on the unlimed than on the limed plots. The lowest pH values for both sections were found on the ammonium sulfate plot, but the soil from the unlimed plot was much more acid than that from the limed plot.

The plot having the lowest pH value throughout the period (11A) shows the highest lime requirement by the Veitch method.

The unlimed nitrate of soda plot (9A) shows a slightly higher pH value than most of the other plots in this section and a lower lime requirement (excepting the unfertilized plot, 7A).

Those plots which gave a pH value of about 7 showed no lime requirement by the Veitch method.

From the above it is concluded that for normal soils a determination of the pH values may be of considerable assistance in arriving at the amount of lime required for general farm crops.

The bi-weekly nitrate determinations showed considerable variation as the season progressed, the lowest period being about the last of July and through August, just after the crop had been removed.

The high results obtained at the two samplings in September are the result, in part at least, of the fertilizers which were applied on September 3 and 4.

The averages for the unlimed plots are, with some exceptions, higher than the averages for the limed plots.

A high content of nitrates does not necessarily mean a good crop yield, there may be other inhibiting factors.

Nitrates may be found in considerable quantities in a soil that is so acid as to practically inhibit growth of ordinary farm crops. Such acidity does not seem to inhibit the growth of crab grass.

There appears to be a slowing down of nitrification with the approach of cold weather (freezing temperature).

A large excess of available nitrates is likely to cause lodging of grain and hay, with a consequent loss in yield.

REFERENCES

(1) Gillespie, Louis, J. 1920 Colorimetric determinations of the hydrogen-ion concentration without buffer-mixtures, with especial reference to soils. *In* Soil Science, v. 9, p. 115–136.

(2) Lipman, J. G., and Blair, A. W. 1920 Field experiments on the availability of nitrogenous fertilizers. N. J. Agr. Exp. Sta. Bul. 260.

(3) Lipman, J. G., and Blair, A. W. 1920 Field experiments on the availability of nitrogenous fertilizers. 1908–1917. *In* Soil Sci., v. 9, p. 371–392.

(4) Lipman, J. G., Prince, A. L., and Blair, A. W. 1921 The influence of varying amounts of sulfur in the soil on crop yields, hydrogen-ion concentration, lime requirement and nitrate formation. *In* Soil Sci., v. 12, p. 197–207.

(5) Schreiner, Oswald, and Failyer, George, H. 1906 Colorimetric turbidity and titration methods in soil investigations. U. S. Dept. Agr. Bur. Soils Bul. 31.

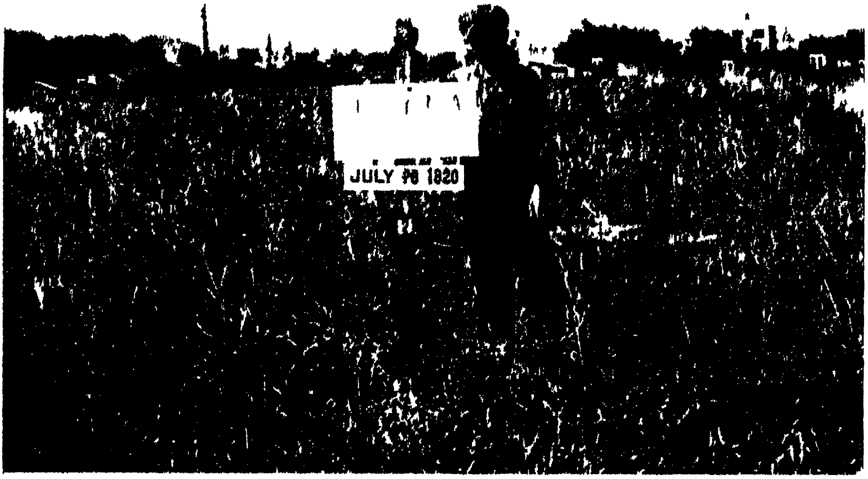


FIG. 1. THE BARLEY ON PLOT 11A, WITHOUT LIME, WAS A FAILURE

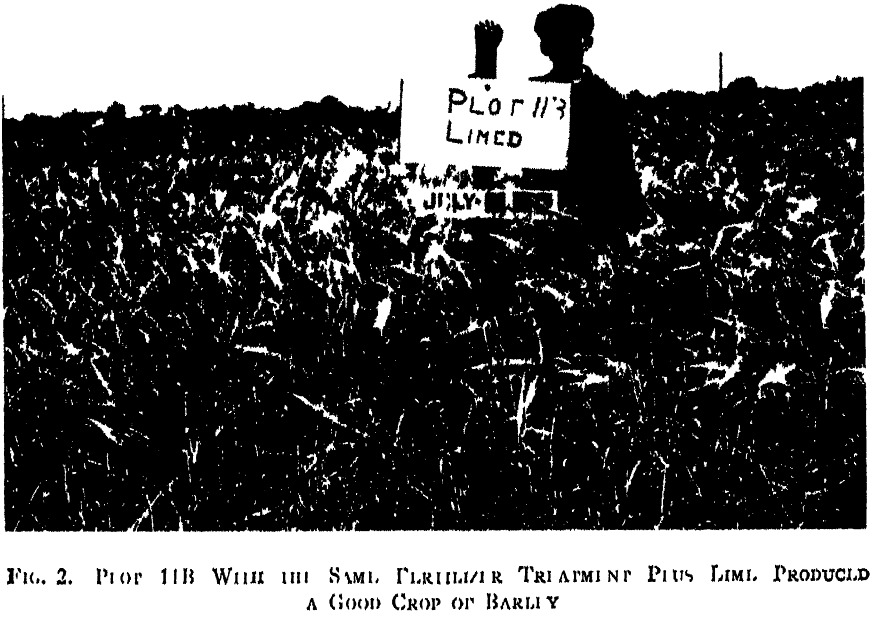


FIG. 2. PLOT 11B WITH THE SAME FERTILIZER TREATMENT PLUS LIME PRODUCED A GOOD CROP OF BARLEY

A POSSIBLE CORRELATION BETWEEN THE FERTILITY OF RICE SOILS AND THEIR TITRATION CURVES

O. ARRHENIUS

From the Treub Laboratory, Buitenzorg, Java

Received for publication August 31, 1921

In Java, as throughout the East, rice-culture plays a very important rôle, because rice is the essential food of the natives. However, the population has grown so rapidly that rice cultivation has not been able to keep pace with it. In order to increase the rice crop, several steps have been taken, such as cultivation by machinery at Selatdjaran, (5) colonization of new land, and improvement of old sawahs.[1] New ways of meeting the situation are well worth trying, and these efforts were made with that end in view.

The rice crop in Java varies from about 100 to 5 pikkols paddi per bouw, with an average of 25 pikkols per bouw for all of Java (that is, 2000 kgm. paddi, or 1000 kgm. rice per hektare). Soils giving less than 25 pikkols per bouw are considered bad.

The causes of bad crops are either external as in the case of too much or too little water, or when the soils are improperly worked, or they are internal, as in the case of bad or unhealthy soil or those becoming easily infected. If one could ascertain the reasons for soils being bad, one could look for a possibility of improving them.

It is not a simple condition with which we are dealing, but a complex of physiological and pathological conditions. Why does a plant become diseased? In most cases it is because the plant lacks strength in one way or another. The basis of our work will be the fact that bad soils cause a weakness of the plant.

Many authors (3, 7) have found that it is possible to classify soils into natural groups according to the hydrogen-ion concentration, expressed in terms of pH values. Since the influence of the hydrogen-ion concentration upon the growth of plants is well known, the possibility suggested itself that some valuable information could be obtained in an attempt to solve the question of how rice soils become bad through an investigation of the hydrogen-ion concentration.

Several soils of different types were used for this work. Ten grams of soil and 50 gm. of water were shaken and filtered. The pH value was determined colorimetrically according to the method of Gillespie (2). The results are given in table 1 which does not show any correlation between the pH values and the various soils, except in two instances. The four most acid soils are bad and the five most alkaline are good. Except for those two groups there is no cor-

---

[1] A sawah is a rice field under water.

TABLE 1

The correlation between the crop productivity of rice soils and their pH values

SOIL NUMBER	LOCATION AND TYPE	PRODUCTIVITY	REACTION
	Madoera, Sapoeloe	Good	8.2
5851	Preanger, Djampangkoelon	Good	8.1
5085	Semarang, Demak III	Good	8.0
	Semarang, Demak Rimboelor	Good	7.8
	Soerabaja, Lebo	Good	7.8
1961	Soerabaja, Modjokasri	Rather bad	7.8
	Madoera, Gedoengan	Good	7.6
	Soerabaja, Kedamean	Bad	7.6
	Soerabaja, Kedamean	Bad	7.6
6747	Cheribon, Kantji	Bad	7.5
5087	Semarang, Dempet	Bad	7.5
5856	Preanger, Djampangkoelon	Bad	7.4
	Soerabaja, Lebo	Good	7.4
1957	Soerabaja, Madjoeagong	Good	7.3
1958	Soerabaja, Djabang Pandan	Good	7.3
	Madoera, Manding	Good	7.3
6204	Preanger, Tjiawi	Bad	7.3
1959	Soerabaja, Djombang	Good	7.2
6751	Cheribon, Nieuw Tersana	Good	7.2
6865	Madicen, Blimbing	Medium	7.2
5086	Semarang, Demak, Godung	Bad	7.2
6866	Madioen, Tjaroeban	Bad	7.2
	Semarang, Meteseh	Bad	7.2
	Semarang	Bad	7.2
6202	Cheribon, Tjiamis	Good	7.1
5857	Pasoeroean, Kerpandjen	Good	7.1
2214	Soerabaja, Lembangboe	Bad	7.1
2358	Pekalongan, Tjomal	Bad	7.1
	Madoera, Baliga	Bad	7.1
	Semarang, Soere	Bad	7.1
2361	Pekalongan, Tjomal	Good	7.0
2360	Pekalongan, Tjomal	Good	7.0
1963	Soerabaja, Tengahan	Medium	7.0
	Batavia, Buitenzorg	Good	6.9
2359	Pekalongan, Tjomal	Good	6.9
5084	Semarang, Bonang	Bad	6.9
	Batavia, Pondoktjina	Bad	6.9
1953	Soerabaja, Madjosari	Good	6.8
	Palembang, Salatjara	Good	6.8
	Batavia, Dejpok	Bad	6.7
1573	Bantam, Sadjpera	Bad	6.7
6203	Preanger, Tjihea	Bad	6.6
	Palembang, Salatjara	Bad	3.8

relation whatsoever. Though these exceptions would seem of little importance they encouraged the author to further investigations.

After determining the titration-curves of various soils and finding their buffer-effect,[2] very distinct correlation between the buffer capacity and the productivity of the soil was found. As seen from the curves (fig. 1, 2 and 3), the soils which act as good buffers are also good soils for rice cultivation. But is the buffer curve a leading property of these soils or is it only of secondary importance?

The rice plant, like many other plants, excretes carbon dioxide, and other substances which act as amphoteric electrolytes, i.e., they can act both as bases and acids. Decaying parts of the plants act in the same way. This was demonstrated by an experiment in which 14 rice-plants about 30 cm. long were transplanted into Erlenmeyer flasks containing 75 cc. of a solution of a known hydrogen-ion concentration. The desired hydrogen-ion concentrations were obtained by adding HCl or KOH so that a series of pH values ranging from 3.0 to 9.0 results. The flasks were than placed in ordinary flower-pots covered with thick paper in which a hole was made, so that only the green parts of the plants were in the light. A corresponding series without plants served as control. At the end of one and two days, the pH volumes were determined in all the flasks. Then the solutions were renewed and the experiment carried on for one more day. The results are shown in table 2. It is obvious that the root excretions neutralized the acids or alkalies in the nutrient solution to a specific point, in this case pH 6.2.

These excretions accumulate in the layer of the soil next to the roots, and this layer then acquires special properties different from those of the surrounding soil. According to work begun by Dr. P. von der Elst on the root development of rice, several facts speak for this hypothesis. When one makes a cut through the soil and the root system of rice, there is seen around the roots a thin red layer of more highly oxidized matter than in the surrounding soil.

In soils with a low buffer content samples were taken partly from the soil surrounding the rice plant, partly from within the root system. The latter samples, when examined, had a lower pH value than the former. In one case a change took place from 7.0 to 6.7, another from 6.9 to 6.8. It therefore seems as though the plant acidifies this root layer, which results, in the case of soils with a low buffer content, in a marked change of hydrogen-ion concentration. Miyake (6) and Daikuhara (1) pointed out that the rice plant does not like even small amounts of acid, and the practical man knows that sawah rice does not thrive in humus soils, i.e., acid soils. Professor Aso of Tokio has kindly shown me a rather good rice soil (50 pikkols per bouw) which was very rich in humus. But its pH value was 7.0. According to a personal

---

[2] "Buffer effect" is taken to mean the soil's capacity to take up various amounts of acid or alkalies without a great change of the pH value. Some soils act as good buffers, some are bad ones.

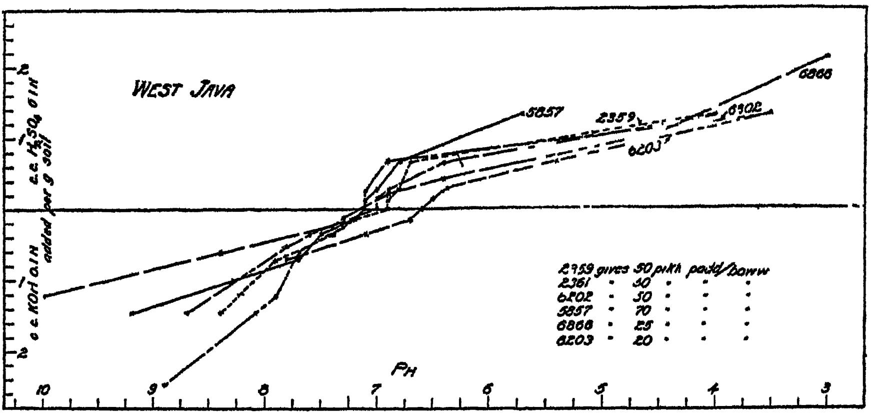


Fig. 1

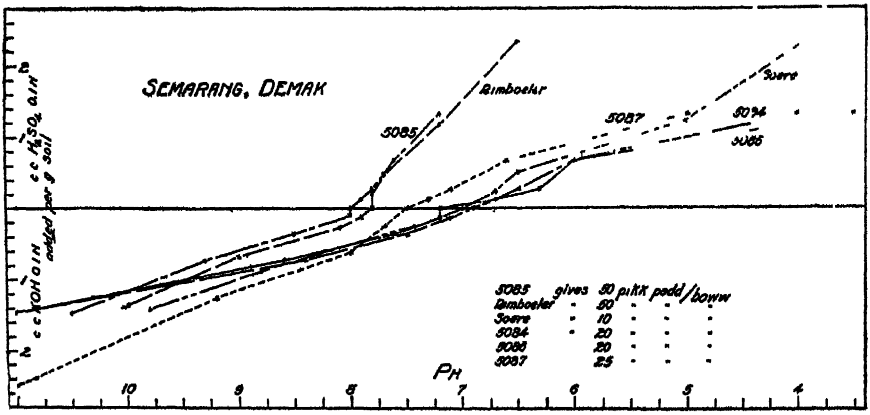


Fig. 2

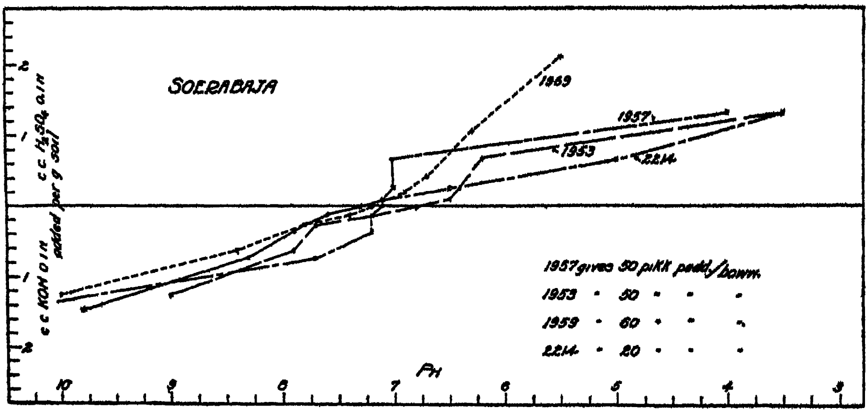


Fig. 3

Correlations Between Crop Productivity and Soil Reaction letter from Professor Aso, green manure, and other materials rich in humus have proven very successful when applied together with lime. It seems as if the rice plant "poisons" the soil by acidifying it, but we see that this holds true only in soils which are weak as buffers or have a too low pH value. This is indeed only a secondary factor. In the acid solutions the aluminum salts are held in solution, while in neutral solutions they are precipitated. The aluminum ion has a very poisonous affect on rice (4). It is more probable that the disease is caused both by the low hydrogen-ion concentration and the aluminum. The rice plant becomes weak in such soils. and therefore falls an easy victim to different diseases.

TABLE 2

Effect of growth of rice plants in well water on its reaction

CULTURE	ORIGINAL SOLUTION			RENEWED SOLUTION
	Initial reaction	After 1 day	After 2 days	After 1 day
Control	3.0	3.0	3.0	3.0
Plants	3.0	4.5	6.2	5.0
Control	4.0	4.0	4.0	4 0
Plants	4.0	5.0	6.2	6.0
Control	5.0	5.0	5 0	5.0
Plants	5.0	6.0	6.2	6.0
Control	6.0	6.0	6 0	6.0
Plants	6.0	6.2	6.2	6.2
Control	7.0	6.9	6.8	7.0
Plants	7.0	6.1	6.1	6.0
Control	8.0	7.5	7.2	7.0
Plants	8.0	6.3	6.2	6.0
Control	9.0	7.8	7.5	8.0
Plants	9.0	6.1	6.2	6.0

What can be done to improve these bad soils? The question is hard to answer after mere laboratory experiments, but some suggestions can be made.

By adding lime, the soil can be made more resistant to acid substances for several years. The best way to apply lime is to put on small quantities at different times. In that case, the lime has a better opportunity to react with the components of the soil and will not be washed away so much by the irrigation water.

A bad soil, for instance the bad Demak, requires an application of about 2.5 tons of lime per hektare. assuming that the soil is worked to a depth of 20 cm. and that the soil, when dried at 100°C., has a volume weight of 1.5 and that it shall then have the same reaction as the good Demak.

However, in order to make the soil good for a longer period of time, a buffering substance must also be added. The best and cheapest is a good green manure crop. I am aware that much is said against humus, for, "Sawah rice can't be grown on soils rich in humus." But nearly all humus soils in Java have an acid reaction: only one out of thirty soils examined was alkaline, and this due to the fact that the place where it was found was rich in lime. It would seem therefore that it is the acid reaction which prevents rice from growing and not the fact that the soil is rich in humus. The soil which professor Aso showed me, mentioned above bears out this conclusion. By adding lime to the humus one can have a good buffer substance of the right reaction.

I take the opportunity of thanking Dr. P. von der Elst, Mr. T. T. White, and Landbouwleeraat Vink for their friendly help in taking soil samples and for their many suggestions.

REFERENCES

(1) DAIKUHARA,—1919 Ueber saure Mineralboden. Bul. Imp. Agr. Centr. Stat. Tokio.

(2) GILLESPIE, L. J. 1920 Colorimetric determination of hydrogen-ion concentration without buffer mixtures with special reference to soils. *In* Soil Sci., v. 9, p. 115–136.

(3) GILLESPIE, L. J., AND HURST, L. A. 1918 Hydrogen-ion concentration—soil type—common potato scab. *In* Soil Sci., v. 6, p. 219–236.

(4) HARTWELL, L. R., AND PEMBER, F. R. 1918 The presence of aluminum as a reason for the difference in the effect of so-called acid soil on barley and rye. *In* Soil Sci., v. 6, p. 259–279.

(5) MEULEN, W. A. VAN DFR 1921 Een bezoch aan hed machinale rijstbedrijf te Selatdjaran bij Palembang. De Waterstaatsingenieur, 1921.

(6) MIYAKE, K. 1916 Toxic action of soluble aluminum salts upon the growth of the rice plant. *In* Jour. Biol. Chem., v. 25, p. 23.

(7) SHARP, L. J., AND HOAGLAND, D. R. 1916 Acidity and absorption in soils as measured by the hydrogen electrode. *In* Jour. Agr. Res., v. 7, p. 123-145.

A TENTATIVE OUTLINE OF THE PLATE METHOD FOR DETERMINING THE NUMBER OF MICRO-ORGANISMS IN THE SOIL

S. A. WAKSMAN

New Jersey Agricultural Experiment Station

AND

E. B. FRED

University of Wisconsin

Received for publication July 20, 1922

At the last meeting of the Society of American Bacteriologists (December, 1921), a group of soil bacteriologists came together in an informal way to discuss problems relating to the desirability of developing standard methods to be used in soil bacteriological work and to bring about a greater coöperation in the investigation of soil bacteriological problems. A committee was appointed, consisting of Dr. S. A. Waksman of the N. J. Agricultural Experiment Station, and Dr. E. B. Fred of the University of Wisconsin.

In view of the fact that the methods used in soil bacteriological work vary with almost every laboratory where that work is carried out, the committee is proposing to examine the various methods with the idea of bringing about a uniformity of the methods leading to a better understanding of the results obtained. Suggestions will be appreciated. Correspondence may be addressed to either member of the committee.

The committee is here proposing a tentative outline of the methods to be used in determining the numbers of microörganisms by the plate method. Every soil bacteriologist is urged to submit these methods to a comparative test with the methods used in his own laboratory and give the committee the benefit of his results and opinions.

I. Culture media.

1. Composition of media for determining numbers of bacteria and actinomycetes.

(a) *Sodium albuminate agar.* 1000 cc. distilled water, 1 gm. dextrose, 0.5 gm. K_2HPO_4, 0.2 gm. $MgSO_4 \cdot 7H_2O$, trace of $Fe_2(SO_4)_3$, 0.25 gm. egg-albumen dissolved in 0.1 *N* NaOH to make alkaline to phenolphthalein, 15 gm. agar.

(b) *Sodium caseinate agar.* Same as albuminate agar, except that 1 gm. of casein dissolved in 8 cc. of 0.1 *N* NaOH is used. The casein should be made according to Hammarsten or Van Slyke method. If nutrose (sodium caseinate) is used, no neutralization is necessary.

(c) *Soil extract agar and gelatin.* 1 kgm. of good fertile soil is heated with 1 liter of tap water for 30 min. in the autoclave, at 15 lbs. pressure. The turbid liquid is then poured off, a little talc is added, and the liquid filtered through a double folded filter paper, to obtain 800 cc. of filtrate. To make up 1 liter of medium, 900 cc. of distilled water, 100 cc. of this extract, 0.05 per cent K_2HPO_4, and 150 gm. of Gold Label gelatin are used. 5 gm. NaCl may be added per liter, to prevent the spread of liquefying colonies. If agar is desired, add 15 gm. instead of the gelatin.

(d) *Other media.*

2. Reaction of media—The reaction of the media should be carefully adjusted to about pH 6.8. During sterilization this medium may become more acid. After last sterilization the pH should be about 6.5.
3. Preparation and sterilization of media.
4. Media for the study of the number of fungi in the soil:

Synthetic acid medium: 1000 cc. distilled water, 10 gm. dextrose, 5 gm. peptone, 1 gm. KH_2PO_4, 0.5 gm. $MgSO_4$, 1000 cc. of distilled water, add acid to adjust pH to 4.0, 25 gm. agar.

II. Sampling of soil and preparation of dilutions:
  1. *Number of soil samples to be taken:* large composite samples of soil thoroughly mixed; at least 2 or 3 mixed samples should be used.
  2. *Number of plates to be used for each soil sample:* at least 5–10 plates for each final dilution.
  3. *Number of colonies allowable per plate (final dilution):* 50-150; the numbers obtained by the final dilutions should check within 10 per cent.

III. Incubation:
  1. *Temperature of incubation:* 28–30°C. except in the case of gelatin.
  2. *Period of incubation:* at least 8 to 10 days for bacteria; 14 days for actinomycetes; 2 to 3 days for fungi (special medium). Incubate plates under bell jar or tin cans to prevent evaporation.

IV. Counting of plates

V. Mathematical interpretation of results

VI. Maximum error to be allowed for an accurate count

VII. Types of colonies on the plate: Molds, Actinomycetes, Yeasts, Chromogenic forms, *Mycoides*, *Fluorescent*, *B. subtilis*, *B. vulgatus*, *B. coli*, etc.

MOVEMENT OF LEGUME BACTERIA IN SOIL[1]

W. C. FRAZIER AND E. B. FRED
University of Wisconsin, Madison, Wisconsin

Received for publication October 21, 1921

A study of the amount and rate of movement of legume bacteria in soil is of practical importance from the standpoint of inoculation. It would at first seem probable that the successful inoculation of most of the legume plants throughout a field would mean the subsequent inoculation of the remaining plants in the field. If this were true, a very light inoculation of a field or of a lot of seed would be almost as effectual as a heavier inoculation. In practice, this has been found untrue, for in field tests where inoculated and uninoculated plants are grown in adjacent rows, the uninoculated plants rarely show nodules. Then, after the nodules have broken up and decayed, there is the question of how much and how fast the legume bacteria will move through the soil.

That the soil is an effective filter for removing organisms has long been established; and the fact is made use of in water purification. This filtering action is particularly noticeable where the soil about a legume is inoculated at the surface with a suspension of the proper legume bacteria. In this case only the upper part of the root system produces nodules. This was observed as early as 1892 by Nobbe, et al. (1) and has been often observed by the writers. These investigators also inoculated sand at a depth of 8 inches and found that only the deeper roots were inoculated. Their conclusion was that legume organisms do not diffuse readily through the soil. Hunter (2) showed that this filtering effect varied with the kind of soil and the depth of the soil.

Kellerman and Fawcett (3) worked with *B. radicicola*, *B. ochroceus* and a *paracolon* organism in sterilized favorable soils saturated with water. They observed that the organisms progressed horizontally at the rate of about 1 inch in 48 hours. In barely moist soils the rate of movement of *B. radicicola* was reduced to about 1 inch in 72 hours. These experiments were conducted at a temperature of 25°C. When the temperature was 10°C. the rate of progress of *B. radicicola* was only about 1 inch in 3 days in saturated soils. The progress was assumed to be due to growth of the organisms.

Ball (4) planted bacteria free alfalfa and bur clover seeds in boxes of sterilized soil. The soil was watered by a tube that ran full length along the bottom of the box to avoid water currents as much as possible. The soils were inoculated at one end of the boxes with a suspension of legume bacteria, made by crushing nodules of alfalfa or bur clover in water. He concludes that *B. radicicola* diffuses through the soil, if the soil has the proper moisture content. He suggests that the movement is due to currents in the soil water, aided by the motility of the organism and its rapid proliferation. He agrees with Kellerman that the rate of movement is about 1 inch in 48 hours. From a group of similar experiments Hunter (2) drew practically the same conclusions: that the movement of legume bacteria in soil is chiefly a result of water currents. He agreed with Kellerman and Fawcett that a high moisture content in the soil was necessary for an appreciable movement of the legume bacteria.

---

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station, Madison, Wisconsin.

In the experiment outlined below the apparatus was modified so as to cut down to a still greater extent the movement of soil water.

Two galvanized iron boxes 24 x 7 x 7 inches were filled with 5 inches of limed yellow sand. Each box was divided longitudinally by a metal wall, the lower part of which was perforated. This divided the box into a main compartment in which the plants were grown, and a small side compartment, used to receive the water or nutrient solution for the soil. This side compartment was also filled with fine sand. Hence all the water before it reached the plants passed through 6 inches of sand and the perforated division wall. Boxes and sand were heated in the autoclave for 2 hours at 15 pounds, pressure. Soybean seeds were treated with mercuric chloride. In box 1 eight mammoth yellow soybean seeds were planted at 6- or 7-inch intervals; and in box 2 nine soybean seeds were planted at similar intervals. Six days later, when the seedlings had just begun to leaf, an area which extended from the end of the box to the stem of the first soybean plant, within the partition, was inoculated with a heavy suspension of soybean bacteria, a mixture of four tested strains. Thus in box 1 the area wet by the inoculating fluid extended 5 inches from the end, in box 2, 6.5 inches from the end.

Thereafter the sand was kept at approximately three-fourths saturation by watering into the smaller side compartment. Thus, the water entered the larger compartment from the side and below, reducing the possibility of the legume bacteria being transported down the length of the box by water currents. Hopkins and Pettit nutrient solution was also added in this way.

Thirty-five days after inoculation the sand was carefully washed from the roots of the soybean plants, with special precautions not to change the arrangement of the roots. As each layer of sand was washed off, the newly uncovered roots were examined for the presence of nodules. In this way the exact position of all the nodules was ascertained; and in particular the position of the nodules farthest away from the place of inoculation was noted. Similarly, after 54 days the sand was washed from the roots of the plants in box 2.

In both cases the roots of the first four plants had nodules, while the roots of the remaining plants had none. The striking thing, however, was that in both boxes all nodule formation had taken place within a sharply defined zone which included the soil within 6.7 inches from the inoculated end of the boxes. Within this 6.7 inches all the roots were well inoculated; beyond the 6.7 inche mark there were no nodules even on roots of the same plant. This is graphically shown in the accompanying photographs of the second, third, fourth and fifth plants in a box. The roots are arranged just as they were in the soil. The roots of the second plant were entirely within this inoculated area and showed an even distribution of nodules. The roots of the third plant were only half within this area and have nodules accordingly. Two roots from the fourth plant passed the top root of the third plant and reached into the inoculated area and had nodules where they entered that area. The roots of the fifth plant were all outside of the area and hence had no nodules.

This would mean then, that the legume organisms had travelled or been carried at the rate of about 0.1 or 0.2 inch per day in this experiment.

This work, then, substantiates that of the investigators cited above, and shows that where the movement of soil water is minimized the rate of movement of the legume bacteria as found is still less than that previously determined.

A field test at this station has demonstrated on a large scale the slow rate of movement of legume bacteria. A small plot of virgin soil on the university farm was planted to Ito San soybeans. The surface of the plot was far from level, with a decided slope towards one end. The soybeans were planted in hills, 3 feet apart in each direction. It was arranged so that inoculated and uninoculated beans were sown in alternate hills. In one row the inoculted seed were planted in the hills of even numbers, while in the adjoining row the odd numbers were planted with the inoculated seed. The diagram shows the scheme followed in planting.

O X O X O X

X O X O X O

O X O X O X

X O X O X O

O X O X O X

X O X O X O

O = Uninoculated
X = Inoculated

Inoculation was accomplished by applying directly to the seed a pure culture of bacteria. The treated seed was shown as soon as possible after inoculation.

About three months after seeding, just as the pods were formed, several plants from various parts of the plot were dug up carefully and examined for nodules. In every case, roots from the inoculated hills were thickly studded with nodules, while those from the untreated hills were free of nodules. Only one exception was found; here the uninoculated roots showed one or two nodules. When examined again two weeks later the effect of inoculation on nodule production was even more pronounced. The untreated plants were without nodules. Although but one experiment has been made, it seems safe to conclude that in soil of this Miami silt loam type, soybean bacteria spread slowly if at all through soil, unless carried by the host plant or by wind, rain, etc.

REFERENCES

(1) Nobbe, F., Schmidt, E., Hiltner, L., and Hotter, E. 1892 Über die Verbreitungsfähigkeit der Leguminosen-Bakterien im Boden. *In* Landw. Vers. Stat., v. 41, p. 137–138.

(2) Hunter, C. A. 1917 The Behavior of Legume Bacteria in Soil. M. S. Thesis, Univ. of Wisc., Madison.

(3) Kellerman, Karl F., and Fawcett, Edna H. 1907 Movement of certain bacteria in soils. *In* Science, n. s., v. 25, no. 647, p. 806.

(4) Ball, O. M. 1909 A contribution to the life history of *Bacillus* (Ps.) *radicicola*. Beij. *In* Centbl. Bakt. (etc.), Abt. 2, v. 23, p. 47–59.

PLATE 1

FIG. 1. SECOND PLANT FROM INOCULATED END OF BOX
FIG. 2. THIRD PLANT FROM INOCULATED END OF BOX

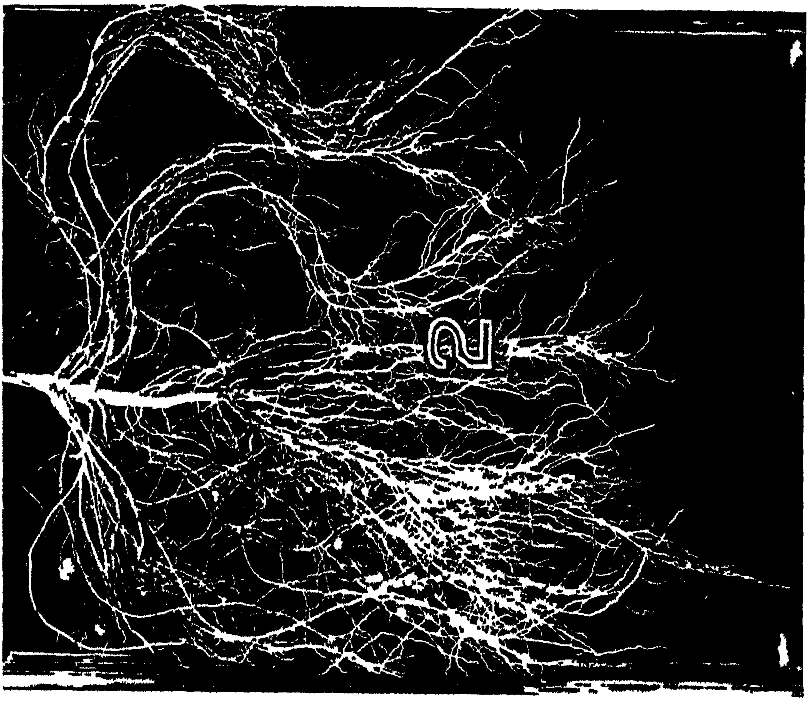


FIG. 2

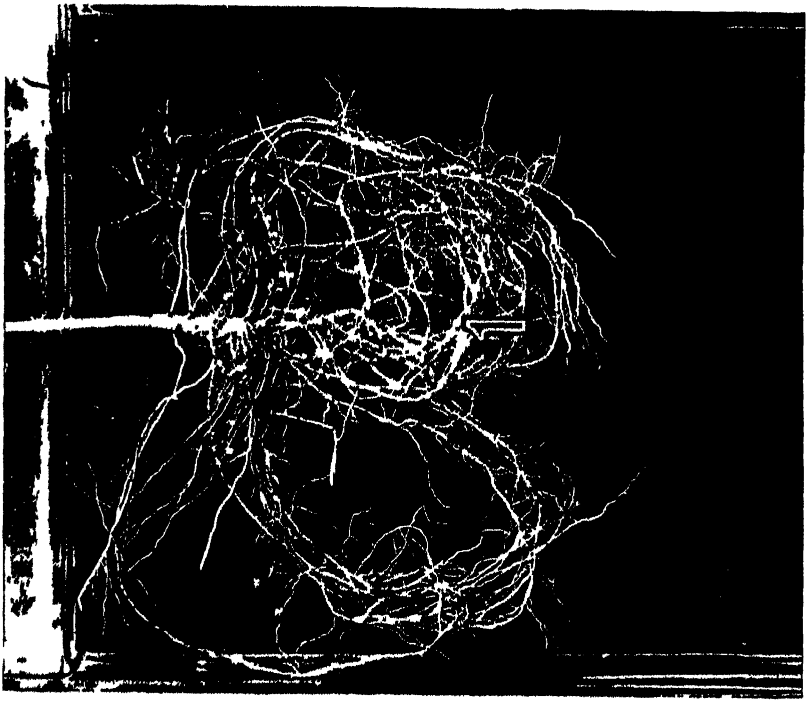


FIG. 1

PLATE 2

Fig. 3. Fourth Plant from Inoculated End of Box
Fig. 4. Fifth Plant from Inoculated End of Box

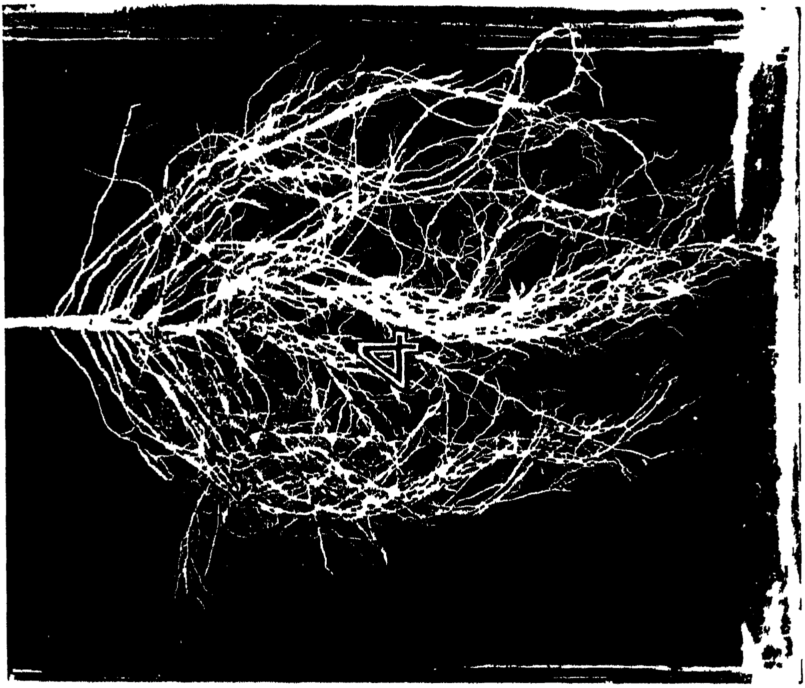


FIG 4

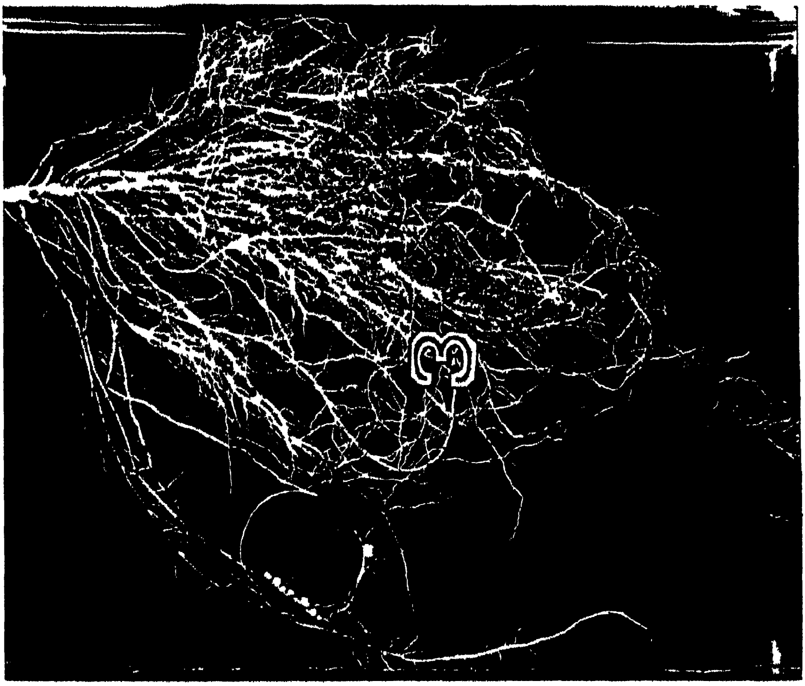


FIG. 3

COMPOSTING ROCK PHOSPHATE WITH SULFUR IN SLIGHTLY ALKALINE CALCAREOUS SOILS[1]

W. RUDOLFS[2]

New Jersey Agricultural Experiment Stations

Received for publication October 18, 1921

INTRODUCTION

The studies made by Lipman, McLean and Lint (10, 11) on the effect of oxidation of sulfur in soils upon the solubility of rock phosphate led to the positive conclusion that the method of composting rock phosphate with sulfur could be made practical and moreover would have an advantage over the old methods of making phosphoric acid rapidly available for plant food.

In continuing their experiments they concluded that mixtures of soil, rock phosphate and sulfur for plants grown in pot cultures compare favorably in value with commercial products. Since then several investigators have conducted experiments in the same field.

The purpose of the work of Lipman and his associates at the start was to produce a compost mixture which could easily be made by farmers. As soon as laboratory experiments are brought to the farm a number of difficulties arise, such as methods of mixing, the optimum water-content of the composts during incubation, proportion of soil, temperature, and the length of time required before the mixture is ready for use. When such general laboratory experiments are being duplicated in the factory still more problems have to be solved, which do not concern the workers in the Agricultural Experiment Stations.

One of the main problems is the time required before the cultures begin to make the tricalcium phosphate available for plant food. McLean found in his best cultures that after 15 weeks small amounts of phosphorus became available. The recently reported experiments by Lipman and Joffe (9) show, however, that under favorable conditions, as an abundance of soil, high initial acidity of the soil used, excess of sulfur, and rather high temperature this time may be considerably reduced. To duplicate these favorable conditions on a large scale a number of difficulties have to be overcome.

Lindet and Bruno (8) doubted that sulfur oxidizing organisms in compost mixtures of rock phosphate, sulfur and a *calcareous* soil could produce sufficient acid to dissolve the phosphates, for "the acid formed would by preference attack the calcium carbonate and not the tri-calcium phosphate." The opportunity has been given to the writer to make a study of these and other problems in France where the greatest part of the soils are calcareous. Later (13) it was found that sulfur organisms would oxidize sulfur in the presence of "alkali" salts.

---

[1] Paper No. 90 of the Journal Series, New Jersey Agricultural Experiment Stations, Department of Soil Chemistry and Bacteriology. This paper will appear in RUTGERS COLLEGE STUDIES, Vol. 1.

[2] Part of a thesis submitted to the faculty of Rutgers College and the State University of New Jersey in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

The writer wishes to express here his thanks to Dr. J. G. Lipman for the opportunity to carry on these studies in France, to Dr. André Hellbronner, formerly chef de laboratoire in the Sorbonne University at Paris for his cordial encouragements throughout the period of investigations, and to Monsieur J. Gavelle, Directeur de la laboratoire de récherches des Établissements Kuhlmann à Paris for the help rendered in analyzing the numerous samples.

PURPOSE OF THE WORK

The work undertaken dealt mainly with the problem of how to reduce the first part of the incubation period in mixtures of soil rock phosphate and sulfur mixtures before the phosphorus becomes available when a *calcareous, slightly alkaline soil* is used, and of reducing the amount of such a soil in the mixture to a point which would make the process practical in the factory.

A study was made of the following factors:

1. Influence of temperature on the activities of the sulfur-oxidizing organisms.
2. Influence of light.
3. Partial sterilization of the mixtures.
4. Effect of stimulants.
5. Initial reaction.
6. Aeration.
7. Replacement of soil by other substances as a source of nitrogen.
8. Reduction of the proportion of sulfur in the mixtures.

METHODS

The composts were made by thoroughly mixing air-dry portions of soil, rock phosphate, sulfur, and other materials used, if not otherwise stated, and were then placed in tumblers covered with glass plates. Each compost was weighed and the water-holding capacity determined on portions of it according to the Hilgard (6) method. Samples were weighed out and the relative acidity, hydrogen-ion concentration and citrate-soluble phosphoric acid determined. The mixtures were then inoculated with soil extract known to contain the sulfur-oxidizing organisms. The compost mixtures were incubated at 30°C. and at laboratory temperature. Daily records taken of the room temperature showed a fluctuation of the temperature between 10°C. and 21°C., the average temperature being 17°C. The composts were kept at 60 per cent of the water-holding capacity throughout the period of incubation. The amount of water lost by evaporation, determined by placing the tumblers on the scale pan, was added once each week to the composts kept at room-temperature and twice each week to the composts incubated at 30°C. Each time, after the water was added, the mixtures were removed from the tumblers and thoroughly mixed to provide for sufficient aeration, and to break up the small aggregates formed.

Citrate-soluble phosphoric acid was determined by sifting the air-dry sample through a 50-mm. sieve. Two grams of this material was finally ground in a cobalt mortar with a total amount of 80 cc. ammonium citrate (400 gm. per liter), decanted into a 200-cc. flask under repeated grinding. The remainder of the material in the mortar was washed into the flasks with distilled water. The stoppered flasks were shaken and left standing for 15 hours at room temperature. They were then kept for 1 hour on a water bath at 40°C., cooled, made to volume, shaken and filtered. An aliquot portion was precipitated with $MgNH_4Cl$, and washed five times with ammonia water (4 per cent). The filter with contents was left standing for a few hours to dry, transferred to a platinum crucible, ignited (placed at a blow flame till weight was constant), cooled and weighed as $Mg_2P_2O_7$.

The relative acidity was determined by transferring an air-dry weighed sample to 200-cc. flasks with 160 cc. of boiling water, thoroughly shaking every 5 minutes throughout a period of ½ hour, cooling flasks and contents, restoring volume and shaking again. After settling, an aliquot was drawn off, boiled to expel carbon dioxide, cooled and titrated with 50 *N* sodium hydroxide. Phenolphthalein was used as indicator and the liquid titrated until a faint pink color remained. The results in the tables, however, are given as 0.5 normal sodium hydroxide.

The water extracts for the determination of the hydrogen-ion concentration were prepared according to the method of Gillespie (5). The hydrogen-ion concentrations as expressed in pH values of the extract were determined by the colorimetric method as recommended by Clark and Lubs (3). Duplicate determinations of the hydrogen-ion concentrations, but not of relative acidity nor of the phosphoric acid were made, except when the results did not agree with the constructed curves for the relation between acidity and available phosphoric acid.

From time to time sulfate determinations were made to check up with the relative acidity.

PLAN OF EXPERIMENT

Several sets of experiments were arranged in a similar way and according to the following plan:

The proportions of the mixtures were:
100 gm. soil
120 gm. sulfur
400 gm. rock phosphate

Soils used were slightly alkaline, calcareous clayey silt loams; the water extract requiring of 2–4 cc. 50 *N* hydrochloric acid per 100 gm. for neutralization and having pH values of 7.1–7.6. These soils were poor in organic matter; and their water-holding capacity was but 24 and 26 per cent.

The flowers of sulfur were from the Italian mines.

The rock phosphate used was Tunisian rock containing 25.99 per cent of total phosphoric acid and 56.79 per cent of total tri-calcium phosphate.

The water-holding capacity of the soil-sulfur-rock phosphate mixtures was 20 and 22 per cent.

EXPERIMENTAL RESULTS

Series I. Influence of light, temperature and peptone

Until the experiments reported below were begun nothing was known about the influence of temperature on the production of available phosphoric acid.

Since then Shedd (16) has reported greenhouse experiments on the availability of phosphoric acid in compost mixtures and he concludes from his study that high temperature exerts a decided influence on the sulfate production and the available phosphorus.

McLean (12) states that when peptone was added to the mixtures "sulfur was largely transformed into sulfites." He laid emphasis on the fact that peat, manure and peptone should not be added to the mixtures for "a compost is more efficient in the absence of large amounts of organic materials." Brown and Gwinn (1, p. 369–389) showed in their experiments that more available phosphoric acid is produced where manure is included in the compost. Ellett and Harris (4) in their recently published work came to the same conclusion. It should be mentioned here that McLean used soils with sufficient organic matter, especially where he used rich greenhouse soils.

In earlier work it was demonstrated by several investigators that various soil types are not alike in their ability to render rock phosphate soluble. At the suggestion of Dr. Lipman compost experiments were carried on in different localities with different soil types. Aside from the "power" of soils to produce sulfates, which according to the results obtained by

Shedd (15), Ellett and Harris (4), and Brown and Kellogg (2, p. 49–111) varies with the soil, the addition of peptone to a soil poor in nitrogenous material could therefore throw light upon the problem of whether or not nitrogen in sufficient quantities is necessary for the activities of the sulfur organisms.

The experimental results of this series consist of the citrate-soluble phosphoric acid made available during an incubation period of 22 weeks, the relative acidity produced, and of the hydrogen-ion concentrations of these mixtures expressed in pH values at definite intervals during this period.

In order to determine what the effect of light on the activities of the sulfur organisms might be, two sets of quadruplicate tumblers were placed on a laboratory table and two similar sets were kept in a dark closet underneath the table. To duplicates of these sets was added peptone equivalent to 3 per cent of the soil mixtures for the purpose of studying the influence of nitrogenous matter. Two other duplicate sets were placed in an incubator at 30°C., of which two mixtures received in addition a similar proportion of peptone. The results obtained at the end of 22 weeks are given in table 1.

TABLE 1

Influence of temperature, light and organic material on the production of acidity and available phosphoric acid in compost mixtures

NUMBER	TREATMENT	INITIAL DETERMINATIONS			AFTER 22 WEEKS		
		Reaction		Citrate-soluble P_2O_5	Reaction		Citrate-soluble P_2O_5
		*alk., cc.**	*pH*	*per cent†*	*cc.°*	*pH*	*per cent†*
1	In darkness, laboratory temperature.	0.04	7.2	0.0	73.3	4.8	0.10
2	In darkness, laboratory temperature, 3 per cent peptone.	0.04	7.2	0.0	220.7	3.0	9.31
3	In light, laboratory temperature.	0.04	7.2	0.0	45.8	4.9	0.31
4	In light, laboratory temperature, 3 per cent peptone.	0.04	7.2	0.0	193.2	3.1	8.72
5	In darkness, 30°C.	0.04	7.2	0.0	132.1	3.2	5.24
6	In darkness, 30°C., 3 per cent peptone.	0.04	7.2	0.0	278.0	3.0	18.92

* Alkalinity expressed in cc. of $0.5N\ H_2SO_4$

† Total P_2O_5 content in mixture was taken as 100 per cent.

° Acidity expressed in cc. 0.5 *N* NaOH required to neutralize 100 gm. air-dry compost.

The relative acidity, hydrogen-ion concentration and available phosphoric acid increased in all cases regularly, which when plotted formed similar curves. The quantities of acids or acid salts increased continuously, but the intensity of the acid as indicated by the pH values remained the same after a certain point was reached, the acid being neutralized by the tri-calcium phosphate. If more sulfur had been applied the strength of the acid produced would presumably have been greater, but since only a small theoretical excess of sulfur was given and the cultures were not carried on till greater amounts of tri-calcium phosphate were made available, the pH values remained at from 2.9 to

3.0. Determinations of hydrogen-ion concentrations made at the end of every week showed slight differences from week to week in pH values, namely, 2.9 to 3.2.

The influence of temperature was very marked throughout the time of experimentation, the higher temperature causing a much higher relative acidity and consequently producing more available phosphoric acid. Even in the mixtures to which peptone was added the accumulation of soluble P_2O_5 was twice as great when the compost was incubated at 30°C. (No. 6) as when they were incubated at room temperature (No. 2).

The nitrogenous material introduced in the form of peptone in this calcareous soil which was poor in nitrogen, had a still greater influence. Culture 5 kept at 30°C. had, after 22 weeks, but 5.2 per cent of the total P_2O_5 made citrate-soluble and had accumulated an acidity equivalent to 132.1 cc. 0.5 *N* NaOH per 100 gm. mixture, while culture 2 kept at room temperature and to which peptone was added showed 9.3 per cent of the total P_2O_5 made available, and an acidity equivalent to 220.7 cc. 0.5 *N* NaOH. This explains at the same time why additions of manure in certain cases, as found by other investigators, render more phosphoric acid available. The sulfur-oxidizing organisms need a certain quantity of nitrogen to carry on their activities. The source of the nitrogen does not necessarily have to be of an organic origin as is shown in another part of this study. When the amounts of nitrogen in the soil are sufficient, additions of nitrogenous materials become detrimental as is pointed out by McLean.

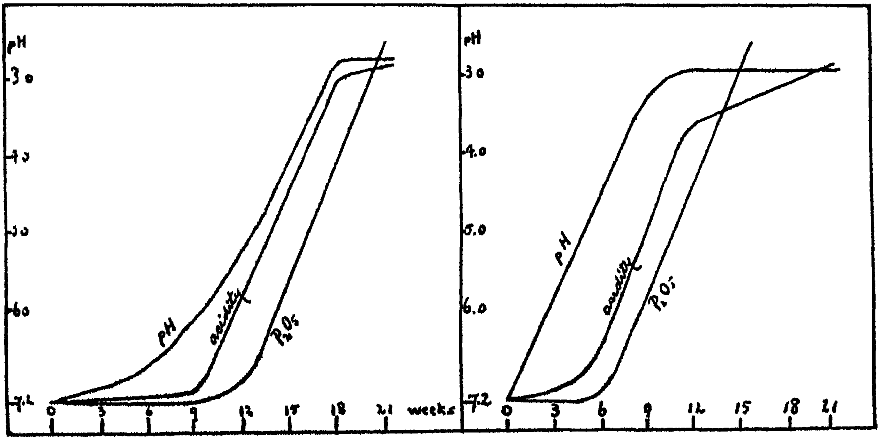


FIG. 1. ROOM TEMPERATURE FIG. 2. 30° CENTIGRADE

Influence of temperature upon the change of hydrogen-ion concentration and accumulation of acidity and available phosphoric acid.

From this series it is evident that calcareous slightly alkaline soil does not inhibit the activities of the sulfur-oxidizing organisms either from attacking the tri-calcium phosphate or transforming it into a soluble form as had been supposed by Lindet and Bruno. In all cultures there was an accumulation of sulfates as expressed in terms of acidity, even in the cultures kept in the light at laboratory temperature and without additions of peptone.

The influence of light was noticeable although not very pronounced. Diffused light in factories might be somewhat detrimental, but presumably very slightly if conditions were more favorable than those prevailing in this experiment.

From a number of cultures, curves were constructed to bring out the relation between the accumulation of acidity, pH values, and quantities of soluble phosphoric acid produced. These relations are shown graphically in figures 1 and 2. The curves in figure 1 are constructed from mixtures incubated at toom temperature, and the curves of figure 2 for the cultures incubated at 30°C. These two sets of curves show clearly the influence of temperature upon the activities of the sulfur-oxidizing organisms. Cultures incubated at room temperature accumulated acidity and consequently changed the hydrogen-ion concentration very slowly during the first weeks, but cultures incubated at 30°C. commenced to change the reaction of the mixtures instantly at the beginning of the incubation period, the rate of accumulation of acidity decreasing in the same way when a certain point is reached both for the cultures incubated at room temperature and for those incubated at 30°C. That part of the curve represents the point at which most of the phosphorus becomes available. The curves of figure 2 show that the amounts of free acid increased with the increase of the temperature.

Series 2. Stimulation

In the work reported by McLean (12) a number of salts and organic substances were added to the compost mixtures in order to determine which salt or substance would exert a stimulating action on the sulfur oxidation processes. He found that all salts used, except, under certain conditions, ferrous sulfate and aluminum sulfate and a combination of the two salts, had no influence on the production of available phosphoric acid. Since sulfuric acid and acid sulfates are produced by the organisms it was thought that small amounts of H_2SO_4 added at the beginning would possibly stimulate their activities.

The soil used in this series consisted of an equal mixture of two different fine calcareous silt loams, which were slightly alkaline, their water extracts having pH values of 7.4 and 7.5.

In one series the sulfuric acid was added before the mixtures were inoculated with the soil infusion and in another series immediately afterwards. The two series checked very closely and the results obtained from the first are given in table 2. At the end of the first week peptone equivalent to 3 per cent of the soil used was added to all series. All mixtures were incubated at room temperature. Determinations of acidity, hydrogen-ion concentrations and citrate-soluble P_2O_5 were made at the end of every three weeks, but only the data obtained at the beginning and at the end of 18 weeks are included as representative figures of this series.

During the first eleven weeks cultures 1 to 7 particularly seemed more or less stimulated by the treatment as compared with the check cultures, but they were gradually overtaken by the checks in acidity accumulation, till after 18 weeks several of the cultures were behind the checks. Cultures 2, 3 and 4, however, had at that time a still higher degree of acidity and also more available phosphoric acid than had the check cultures. From these and other experiments, the conclusion can be drawn that when a fine calcareous silt loam is used, H_2SO_4 exerts a stimulating influence during the first part of the incubation period if quantities of approximately 7 to 10 liters (52° Bé) per ton are used, but depresses the activities of the sulfur-oxidizing organisms when larger amounts are added.

Several salts were tried out in the earlier work by McLean as possible catalytic agents. Some organic acids and two different acid salts which suggested themselves for several reasons had not been employed. As organic acid for our experiment acetic acid, which could possibly lower the alkalinity of the mixtures, was chosen and as acid salts, sodium bi-carbonate as a possible source of carbon di-oxide and sodium bi-sulfite.

TABLE 2

Influence of sulfuric acid in compost mixtures when a fine calcareous alkaline soil is used

NUMBER	1.0 *N* H_2SO_4 APPLIED PER 100 GM. OF MIXTURE	INITIAL DETERMINATIONS			AFTER 18 WEEKS		
		Reaction		Citrate-soluble P_2O_5	Reaction		Soluble P_2O_5
	cc.	*alk., cc.**	*pH*	*per cent*†	*cc.*°	*pH*	*per cent*†
1	1.8	0.05	7.1	0.00	140.4	3.6	9.65
2	3.8	0.04	7.1	0.00	165.5	3.5	10.40
3	5.8	0.06	7.1	0.00	197.3	3.4	12.10
4	6.8	0.01	7.0	0.00	160.2	3.2	11.41
5	7.8	0.00	6.9	0.10	126.1	3.5	9.15
6	9.8	0.01	7.0	0.08	90.6	3.6	8.80
7	11.8	0.00	6.8	0.93	87.4	3.6	7.17
8	13.8	0.02	7.0	1.13	78.4	3.6	4.65
9	None	0.04	7.2	0.00	147.8	3.3	9.98

* † ° Same as table 1.

These constituents were added in small quantities to mixtures as previously described, but no additions of sulfuric acid were made. Treatment of the mixtures and the results obtained after 12 weeks are reported in table 3.

Acidity and hydrogen-ion concentrations were determined at the end of every two weeks, but are not reported here since these data were merely used in checking up the procedure of the process of sulfate production.

The cultures to which acetic acid was added ran in duplicate very inconsistently, but no stimulation could be noticed.

There was a gradual increas of acidity in all mixtures, but although a high relative acidity was reached in cultures 5, 6, and 7, the intensity of the acid produced as indicated by the low hydrogen-ion concentration seemed less. Nevertheless, a marked stimulating effect was to be noticed throughout the entire period of the experiment in the production of acidity and citrate-soluble phosphoric acid. This might have been due to a production of CO_2 (probably needed by the sulfur organisms for greater acitivity) when other bacteria were depressed to a high degree in these acid media.

The cultures to which sodium bisulfite in solution was added as well as the cultures with acetic acid were behind the checks. The influence of $NaHSO_3$ was depressing rather than stimulating for these soil types.

Two series of cultures were made up of the same constituents as described under series 1, and kept at toom temperature, one in light and the other in darkness. Two other series at 30°C. were similarly treated. One tumbler in each series was treated with varying amounts of sulfuric acid (see table 2), another with sulfuric acid and a mixture of .02 per cent $FeSO_4$ and .02 per cent $Al_2(SO_4)_3$, and still another with the iron salts but no sulfuric acid. From the data obtained it was evident that if stimulation was caused by the iron salts it was not great enough to be out of the range of the experimental error.

TABLE 3

Influence of acetic acid and sodium bicarbonate and sodium bisulfite in composts kept at room temperature

NUMBER	TREATMENT	INITIAL REACTION		AFTER 12 WEEKS		
				REACTION		Citrate-soluble P_2O_5
		*alk., cc.**	*pH*	*cc.°*	*pH*	*per cent†*
1	None	0.04	7.2	103.6	3.3	6.69
2	2 cc. 1.0 *N* CH_3COOH	0.02	7.1	71.4	4.8	0.83
3	6 cc. 1.0 *N* CH_3COOH	0.02	7.0	75.8	4.1	6.34
4	10 cc. 1.0 *N* CH_3COOH	0.01	7.0	73.0	4.1	4.32
5	0.01% $NaHCO_3$	0.06	7.3	187.9	4.8	13.94
6	0 02% $NaHCO_3$	0.05	7.2	186.9	4.7	11.43
7	0 03% $NaHCO_3$	0.05	7.2	183.4	4.7	12.05
8	0.10% $NaHSO_3$ (40-% sol.)	0.04	7.3	81.1	5.0	5.10
9	0.20% $NaHSO_3$ (40-% sol.)	0.04	7.3	82.4	5.0	6.01
10	0.30% $NaHSO_3$ (40-% sol.)	0.04	7.3	84.1	5.2	4.70
11	0.50% $NaHSO_3$ (40-% sol.)	0.04	7.3	70.3	5.3	4.52
12	None	0.04	7.1	104.4	3.4	7.01

* † ° Same as table 1.

Series 3. Partial sterilization.

It had been shown in the experiments of Lipman, McLean and Lint (11) that sterilization of the mixtures was detrimental to the production of available phosphoric acid. They therefore drew the conclusion that sulfur oxidation was a biological process. No attempt was made to study the influence of partial sterilization by means of salts or acids. The investigations of Russell (14) with soils treated with antiseptics showed an increase in bacterial numbers as the result of improvement of the soil medium. This worker used carbon disulfide and came to the conclsion that although the bacterial numbers were increased, partial sterilization did not improve the bacterial flora. This was shown by the fact that the old flora, when reintroduced into partial sterilized soil, attained higher numbers and effected more decomposition than the new flora. Partially sterilized soil plus 0.5 per cent of untreated soil, or an unfiltered aqueous extract of untreated soil, soon contained higher bacterial numbers per gram and accumulated ammonia at a faster rate than partially sterilized soil alone. Truffaut (17) concluded after repeated trials that calcium sulfide was an exceptionally good means of partial sterilization. Hutchinson (7) studied the effect of caustic lime for partial sterilization as practical means and obtained good results. Many other investigators worked with different kinds of antiseptics like phenol, naphthaline, toluene, etc., which seemed less suitable for the purpose of this study. The purpose of partial sterilization in the case of the studies at hand would be to suppress undesirable bacterial growth in the cultures and thereby favoring the development and perhaps multiplication of the sulfur-oxidizing organisms.

On account of the relatively high calcium content of the soils used and the abundance of calcium in the rock phosphate used, and in view of the necessary production of acidity, caustic lime was not used. The excellent results obtained by Truffaut in the treatment of soil with calcium sulfide as a means of partial sterilization led the writer to conduct a series of experiments in which the cultures received different amounts of calcium sulfide. In these preliminary studies it was brought out that pure calcium sulfide and the technical product in H_2SO_4 did not produce satisfactory results. The acidity obtained in compost mixtures after 6 weeks of incubation with a neutral garden soil (incubated at 28°C.) was:

Nothing added,	98.4 cc. 0.5 *N* NaOH per 100 gm. mixture.
CaS added,	alkaline.
CaS+ H_2SO_4 added,	12.1 cc. 0.5 *N* NaOH per 100 gm. mixture.

For the partial sterilization studies, the same soil mixtures and the same proportions of sulfur and rock phosphate were used as in series 2. The compost mixtures were kept at room temperature throughout the incubation period. The results obtained with different amounts of NaCl per 100 gm. of mixture are recorded in table 4.

From these data it is readily seen that NaCl added in small quantities depressed the growth and activities of the sulfur oxidizing organisms considerably. Even where but .01 per cent NaCl was added the production of relative acidity after 13 weeks was less than in the check cultures and the available phosphoric acid was less than half the amount produced if compared with the cultures which received no additions of NaCl. Incidentally it was shown that soils secured near the sea coast and containing small amounts of chlorides were not suitable for rapid production of acidity and available P_2O_5. Bacterial counts showed that although fewer species of soil bacteria were present and smaller numbers existed, the sulfur-oxidizing organisms were not favored by their absence. Influence of chlorides on sulfur oxidation by microörganisms was also noted in studies with black alkali soils from California (13).

The same soil-sulfur-rock phosphate mixtures were used for cultures to which 0.1 N HCl was added. Additions varied from 4.5 cc. to 88 cc. per 100 gm. mixture. The compost mixtures were incubated for the first 6 weeks at 30°C. and then placed in a dark cupboard at room temperature. Water was added as usual and the mixtures stirred at the end of each week. Relative acidity and pH values were determined every time when stirred. Most of the free acid had been neutralized by the tri-calcium phosphate, but according to the low pH values considerable free acid was still present at the start of the experiment, especially cultures 9 and 10, as recorded in table 5.

TABLE 4

Influence of partial sterilization on the production of acidity and available P_2O_5 in compost mixtures incubated at room temperature

NUMBER	TREATMENT WITH NaCl	INITIAL REACTION		AFTER 13 WEEKS		
				REACTION		Citrate-soluble P_2O_5
	per cent	*alk., cc.**	*pH*	*cc.°*	*pH*	*per cent*†
1	0.01	0.04	7.2	65.6	3.9	1.82
2	0.02	0.04	7.2	57.2	4.9	trace
3	0.03	0.04	7 3	55.7	4.9	0.83
4	0.01 9 cc. 0.1 N HCl added	0.08	7.3	55.4	4 9	trace
5	Check	0 04	7.2	79 0	3.4	4.15

* † ° Same as table 1.

TABLE 5

Compost mixtures with additions of varying amounts of HCl for partial sterilization

NUMBER	0.1 N HCl ADDED PER 100 GM. MIXTURE	INITIAL DETERMINATIONS			AFTER 14 WEEKS		
		REACTION		Citrate-soluble P_2O_5	REACTION		Citrate-soluble P_2O_5
	cc.	*cc.°*	*pH*	*per cent*†	*cc.°*	*pH*	*per cent*†
1	4.5	0.04	6.8	0.00	66 9	4.9	2.36
2	9	0.10	6.6	0 00	68.4	4.8	2.13
3	14	0.10	6 6	0 00	68.0	4.8	0.77
4	18	0.16	6 7	0.00	64.4	4 8	0.76
5	28	0.12	6.7	0.00	*lost*	...	...
6	38	0.15	6 7	0.00	27.2	5.6	0.61
7	48	0.16	6.7	0.00	20.9	5.8	0.68
8	58	0.50	6.2	0.00	10.1	5.8	0.46
9	68	6.31	5 3	0.68	5.3	6.4	0.19
10	88	7.70	5.2	0.40	5.5	6.4	0.00
11	check	alk.* 0.04	7.3	0.00	104.2	3.2	5.22

* † ° Same as table 1.

After one week of incubation the pH values had gone up and approached the neutral point. There was but slight change at the end of the third week and culture 8 was still slightly alkaline. At the end of 14 weeks all cultures to which HCl was added were far behind the check cultures, in relative acidity as well as in available P_2O_5. Bacterial activities had been very slight in cultures 9 and 10, and the small amounts of P_2O_5 available at the beginning of the experiment were transformed into insoluble phosphates. Even where but 4.5 cc. 0.1 *N* HCl per 100 gm. mixture was added it proved strongly detrimental. The results obtained confirmed the conclusions drawn from the previous experiment that chlorides are detrimental to the activities of the sulfur-oxidizing organisms.

TABLE 6

Influence of varying amounts of soil on the production of acidity and soluble P_2O_5 in composts containing 120 gm. sulfur and 400 gm. rock phosphate, incubated at 30°C.

NUMBER	QUANTITY OF SOIL USED	INITIAL REACTION		AFTER 15 WEEKS		
				REACTION		Citrate-soluble P_2O_5
	gm.	*alk., cc.**	*pH*	*cc.*°	*pH*	*per cent*†
1	25	0.08	7.2	166.6	2.9	9.12
2	50	0.08	7.2	193.6	2.9	12.90
3	75	0.08	7.3	199.3	2.9	13.82
				After 11 weeks		
4	80	0.08	7.3	227.7	2.9	14.10
5	90	0.07	7.3	232.7	2.9	14.00
6	100	0.08	7.3	216.6	2.9	13.92

* † ° Same as table 1.

Series 4. Replacement of soil by other substances as a source of nitrogen

In his studies McLean (12) came to the conclusion that 100 parts of soil, 120 parts of sulfur and 400 parts of rock phosphate would be the most economical combination for the production of available phosphoric acid. All investigators used great quantities of soil, based on the work of McLean. Some of them increased the amounts of soil or added manure. No attempt had been made to reduce the quantities of soil and replacing it by other material as sources of nitrogen except in the studies of pure cultures which were made in the laboratory of the New Jersey Agricultural Experiment Stations, which studies were made mainly during the absence of the writer.

For practical purposes a great bulk of soil in the mixtures is undesirable. The high cost of handling and transportation are regarded as considerable items, aside from the problems of mixing and storing.

A series of experiments was conducted with a rather rich, slightly alkaline, calcareous garden soil. All mixtures were placed in an incubator at 30°C.

Table 6 includes a part of the data secured.

Determinations of citrate soluble P_2O_5 made after 7 weeks showed that cultures 4 and 5 had made more phosphoric acid available than the cultures with 25 parts of soil after a period of 15 weeks. Cultures with 80 or more parts of soil had as much or more available P_2O_5 in 11 weeks as cultures with 75 or less parts of soil in 15 weeks. The difference in relative acidity between cultures with 80 parts of soil or more at the end of 11 weeks was very small. It seemed therefore, that 80 parts of this soil was sufficient from which to expect good results.

From preliminary experiments conducted during this time at the Pasteur Institute and from previous experiments with peptone the conclusion was drawn that the activities of the sulfur-oxidizing organisms depended largely on sufficient available nitrogen. During the progress of the work a more concentrated culture of strong sulfur-oxidizing organisms for inoculation had been secured and was used in the experiments recorded in table 7.

TABLE 7

Influence of ammonium sulfate when the soil is reduced to 10 parts, in a mixture of 100 parts of sulfur and 400 parts of rock phosphate

NUMBER	TREATMENT	INITIAL REACTION		AFTER 11 WEEKS		
				Reaction		Citrate-soluble P_2O_5
		cc.*	pH	cc.*	pH	per cent†
1	None. Incubated at 30°C.	0.40	6.8	227.1	3.0	18.72
2	0.2 per cent $(NH_4)_2SO_4$ added at start. Incubated 30°C.	0.48	6.8	242.7	3.0	22.22
3	0.1 per cent $(NH_4)_2SO_4$ added at start. 0.1 per cent added by stirring. Incubated at 30°C.	0.50	6.8	241.3	3.0	21.90
4	None. Inc. at room temperature.	0.40	6.8	175.7	3.0	16.86
5	0.2 per cent $(NH_4)_2SO_4$ added at start. Inc. at room temperature.	0.50	6.8	172.9	3.0	16.18
6	0.1 per cent $(NH_4)_2SO_4$ added at start. 0.1 per cent added by stirring. Inc. at room temperature.	0.50	6.8	183.1	3.0	16.98

*† Same as table 1.

A mixture of 10 parts of soil, 100 parts of sulfur and 400 parts of rock phosphate was used. It had previously been found that the quantity of sulfur could be reduced to 100 parts, as reported in series 5.

The 10 gm. of soil were of a rich garden soil used in the experiments reported in the previous table. In addition, ammonium sulfate equivalent to 0.2 per cent of the mixture, was given to the cultures 2 and 4 at the beginning, and ammonium sulfate equivalent to 0.1 per cent of the mixture to cultures 3 and 6, while another 0.1 per cent was added to cultures 3 and 6 stirred into during the first 6 weeks.

The available figures show that the compost mixtures to which ammonium sulfate was added and which were incubated at 30°C. made 3.0, 6.0 and 3.2 per cent, respectively, more phosphoric acid available after 11 weeks than the ones with no additions of ammonium sulfate. The mixtures placed at room temperature, however, were all practically alike after this incubation period. It seemed therefore, that enough nitrogenous matter was available up to a certain point. After this point is reached the nitrogen supply seems to be insufficient to keep the organisms at maximum production. Ammonium sulfate furnishes their needed nitrogen. From other experiments (some of which are reported in table 10) it is known that if the quantities of a calcareous soil are reduced to 10 parts or less, sulfur oxidation by the organisms is very slow if not inhibited. It is interesting to note that in the case of these mixtures the influence of temperature was far less pronounced than in the experiments reported in an earlier series. A part of the cultures placed at room temperature were taken away and analyzed at the end of 18 weeks. They had not been stirred during the last 7 weeks and evaporation losses were restored only twice. At the end of this period 27.6 per cent P_2O_5 was available in the mixtures without ammonium sulfate and 34.1 per cent P_2O_5 available in the mixtures to which ammonium sulfate was added.

A part of this relative rapidity of oxidation was attributed to the fact that the small amount of soil did not hinder the production of free sulfuric acid and that the ammonium sulfate added resulted in a slightly acid medium at the beginning of the experiments.

Series 5. Reduction of quantity of sulfur

In the commercial methods of making acid phosphate the proportions of sulfuric acid (52° Bé) and rock phosphate used are approximately equal. The amounts of sulfur employed in composting experiments have usually been larger than the amounts of sulfur used in the commercial methods. If smaller quantities were employed the available phosphoric acid decreased. From a commercial point of view it is interesting to know the results obtained by using these smaller quantities, namely from 20 to 22 per cent of sulfur, especially where the cost of sulfur is high. An excess of sulfur would inhibit composting as might be done in countries where sulfur can be obtained at a comparatively low price. Experiments were made with a soil-rock phosphate mixture in which the amounts of sulfur were reduced to 50 per cent of the original amounts used by McLean and which was pronounced by him as the most economical for farmers. The soil used was a slightly alkaline garden soil containing apparently sufficient nitrogen for the sulfur-oxidizing organisms to produce good results. The compost mixtures were incubated at 30°C. and a part of the results obtained are given in table 8.

From the reported data it is obvious that less sulfur than the theroretical amounts necessary to transform the total phosphoric acid to a soluble form are impractical. It may be seen from the data in this table that a quantity of about the theoretical amount is sufficient to warrant good results. One hundred grams of sulfur, 100 gm. of soil and 400 gm. of rock phosphate gave as good results as 110 gm. of sulfur with the same proportional amounts of soil and rock phosphate, and nearly as good as when 120 gm. of sulfur was employed. When the sulfur was reduced too far the available phosphoric acid decreased accordingly.

TABLE 8

Influence of reducing quantity of sulfur on the production of acidity and available phosphoric acid in composts of 100 parts of soil and 400 parts of rock phosphate incubated at 30°

NUMBER	AMOUNT OF SULFUR USED	INITIAL REACTION		AFTER 18 WEEKS		
				Reaction		Citrate-soluble P_2O_5
	gm.	*cc.*°	*pH*	*cc.*°	*pH*	*per cent*†
1	120	0.4	6.9	266.0	2.9	25.0
2	110	0.4	6.9	247.0	3.0	24.4
3	100	0.4	6.9	247.2	3.0	24.2
4	90	0.4	6.9	246.0	3.0	24.0
5	80	0.4	6.9	213.0	3.0	18.2
6	70	0.4	6.9	204.2	3.0	16.1
7	60	0.4	6.9	201.0	3.1	13.2

° † Same as table 1.

Series 6. Initial reaction

To make a fair test as to whether or not initial reaction would have any influence, 100 parts of an alkaline garden soil, 100 parts of sulfur and 400 parts of rock phosphate were composted and treated at the beginning of the experiment with different amounts of sulfurous acid in some cases, and in other cases sulfurous acid was added to the mixtures when they were stirred for aeration. From the preliminary experiments it was concluded, that approximately 13 cc. sulfurous acid of a strength that will neutralize the same volume of normal sodium hydroxid added per 100 gm. mixture was best suited for the purpose. The mixtures were incubated at room temperature and at 30°C. The data secured are reported in table 9.

The influence of sulfurous acid on the mixtures is very obvious. Although the influence after 15 weeks on the mixtures incubated at 30°C. is not so pronounced as on the mixtures incubated at room temperature, still considerably more phosphoric acid was made available than in the check cultures. The mixtures incubated at room temperature to which sulfurous acid was added at the beginning showed up very favorably in comparison with culture 1 which received no sulfurous acid and was incubated at 30°C. It should be remarked here that a strong culture was used for inoculation similar to that mentioned under series 4. The addition of sulfurous acid by stirring into the mixtures at the times of aeration proved not to be so effective in each case, showing that possible oxidation of sulfurous acid has but little, if any, effect. Since these studies were mainly concerned with reducing the first part of the incubation period before sufficient free acid or acid salts are produced to made P_2O_5 available, the experiments were not continued.

TABLE 9

Influence of initial reaction, using sulfurous acid, on the production of acidity and available phosphoric acid

NUMBER	TREATMENT PER 100 GM. MIXTURE	INITIAL DETERMINATIONS			AFTER 15 WEEKS		
		Reaction		Citrate-soluble P_2O_5	Reaction		Citrate-soluble P_2O_5
		cc. alk.	*pH*	*per cent*	*cc.°*	*pH*	*per cent*
1	None (30°C.)	0.4*	7.3	0.00	211.4	3.0	12.66
2	13 cc. sulfurous acid added by stirring (30°C.)	*acid* 0.4°	6.6	0.00	231.8	3.0	14.12
3	13 cc. sulfurous acid added at start (30°C.)	1.4	6.0	trace	247.6	3.1	17.94
4	None (room temperature)	*alk.* 0.4	7.3	0.00	91.2	3.4	3.08
5	13 cc. sulfurous acid added by stirring (room temperature)	*acid* 0.4	6.6	0.00	194.7	3.1	11.89
6	13 cc. sulfurous acid added at start (room temperature)	1.4	6.0	trace	218.5	3.0	15.38

* † ° Same as table 1.

In a recent publication Lipman and Joffe (9) report that initial reaction is of no advantage when sulfuric acid is used. The mixtures worked with by these investigators had however a relatively high hydrogen-ion concentration at the start, a pH value of 5.4, before additions of sulfuric acid were made. When an alkaline soil is used, the increase of acidity from pH 7.3 to 5.8 requires considerable time, because of the fact that the organisms present have to start with an unfavorable medium. The time required for changing the hydrogen-ion concentration from pH 5.8 to pH 3.2 in such mixtures is usually less than that required for the change from pH 7.3 to 5.8, although the actual acidity produced is much greater in the case of the former. Since these investigators used a decidedly acid medium in their experiments, it is possible that the cause for finding no advantage in lowering the pH values through additions of H_2SO_4, might be found in the initial reaction of the mixtures.

Series 8. Aeration

One of the conclusions drawn by McLean (12) is that the question of aeration should receive foremost consideration. He states that the results obtained would make it appear that the microörganisms which oxidize sulfur are largely aerobic, and hence require an abundant supply of oxygen. The experiments of Shedd (16) show that the stirring of the mixtures had considerable influence, and he concludes that thorough aeration is one of the conditions which promotes most rapid reaction.

Two series of experiments were conducted for the purpose of testing the influence of aeration. The first series was composed of 80 parts of rich alkaline garden soil, 100 parts of sulfur and 400 parts of rock phosphate. To the mixtures were added different ingredients and the cultures placed in a dark cupboard at room temperature. The cultures were divided into two sets. One set was stirred twice a week by removing the compost from the glasses and mixing thoroughly, while the other set was stirred in the same way at the end of 6 weeks when a sample was taken for analyses. Moisture contents were kept at the optimum by weighing at the end of every week. Although all tumblers were kept covered with glass plates, the amounts of water lost from the stirred cultures were far greater than from the cultures which were not removed from the tumblers. The results obtained after 12 weeks are reported in table 10.

In this series of cultures the same was found as in earlier series regarding the influence of sulfurous acid. Sulfurous acid caused considerable stimulation of bacterial activities in the stirred and unstirred mixtures, resulting in relatively higher acidity and greater availability of P_2O_5 as compared with the checks.

Sodium bicarbonate added in solution to this rich soil compost did not stimulate the activities of the sulfur oxidizing organisms but seemed to retard them. This would indicate that the conclusion drawn for the soil poor in organic material and consequently poor in carbon dioxide production was right. There was present in this soil apparently sufficient CO_2 for the work of the organisms and an addition of a CO_2 source had no influence.

Acetic acid proved to be detrimental in this soil and a combination of 0.02 per cent aluminum sulfate and 0.02 per cent ferrous sulfate had no influence. These two salts had no influence upon the activities of the organisms in any of the calcareous soils used.

All stirred mixtures were considerably in advance as compared with the unstirred mixtures. The conclusion drawn by McLean and Shedd that an abundance of oxygen favored sulfur oxidation seemed right. It was thought therefore, that a still greater abundance of oxygen would help accelerate the reaction still more. An apparatus was made in such a way as to have a small continuous stream of moist air running thru the cultures. A diagram of the apparatus used is given in figure 3.

As a soil compost mixture, 10 parts of a neutral calcareous soil, 100 parts of sulfur, and 400 parts of rock phosphate was used. To replace the bulk of the soil a number of cultures received 0.2 per cent ammonium sulfate. The water-holding capacity of this mixture was 22 per cent.

To some of the cultures 10 cc. sulfurous acid in addition was given, while others received the same amount of sulfurous acid at the time of stirring for aeration. Some of the mixtures through which air was running received a similar amount of sulfurous acid at the beginning and others by means of moist air which went through a wash bottle containing the calculated amounts of weak sulfurous acid per 100 gm. mixture.

TABLE 10

Influence of aeration in the presence of different ingredients on the production of acidity and citrate soluble phosphoric acid

NUMBER	TREATMENT	INITIAL REACTION		AFTER 12 WEEKS		
				Reaction		Citrate soluble P_2O_5
	Stirred twice a week					
		cc.	*pH*	*cc.*°	*pH*	*per cent*†
1	None	alk. 0.18*	7.2	31.3	4.3	2.27
2	14 cc. H_2SO_4	alk. 0.18	7.2	54.5	3.9	5.96
3	13 cc. sulfurous acid	acid 0.46°	6.6	137.0	3.2	10.40
4	0.01 per cent $NaHCO_3$	alk. 0.04	7.1	20.3	5.4	1.51
5	6 cc. 1.0 *N* CH_3COOH	alk. 0.14	7.0	5.7	5.6	0.00
6	0.02 per cent $Al_2(SO_4)_3$ and 0.02 per cent $FeSO_4$	alk. 0.18	7.2	27.4	4.1	1.89
	Stirred once every 6 weeks					
7	None	alk. 0.18	7.2	8.2	5.5	trace
8	14 cc. H_2SO_4	alk. 0.18	7.2	44.4	3.9	4.08
9	13 cc. sulfurous acid	acid 0.46	6.6	71.6	3.5	6.51
10	0.01 per cent $NaHCO_3$	alk. 0.04	7.1	4.7	5.7	0.00
11	6 cc. 1.0 *N* CH_3COOH	alk. 0.14	7.0	1.9	5.9	0.00
12	0.02 per cent $Al_2(SO_4)_3$ and 0.02 per cent $FeSO_4$	alk. 0.18	7.2	9.3	5.4	trace

*† ° Same as table 1.

The mixtures through which air was running were taken out of the containers and thoroughly mixed at the end of every two weeks. The cultures in the tumblers were stirred once at the end of each week. Preliminary experiments were made to determine the amounts and rate of air flowing through the mixtures. It was concluded best to use approximately 1 liter of air per hour per 500 gm. of mixture. All mixtures were kept at room temperature. Acidity and hydrogen-ion concentration determinations were made every two weeks, and the soluble phosphoric acid at the end of 14 weeks. The results are recorded in table 11.

While the difference between stirred and unstirred mixtures was considerable, the difference between aerated and stirred composts was still greater. Here, however, the difference was in the opposite direction. All aerated mixtures produced not only less relative acidity, but the available posphoric acid was nil in the aerated cultures (no. 1–4) after 14 weeks. The aerated cultures showed some activity after two weeks but this activity decreased as time went on. In the case of number 4 a part of the measured acidity in all probability was free sulfurous acid which went through, as indicated both by titration and hydrogen-ion concentration. Every 4 weeks moisture determinations were made on the mixtures. These determinations showed that the optimum moisture content was very constant.

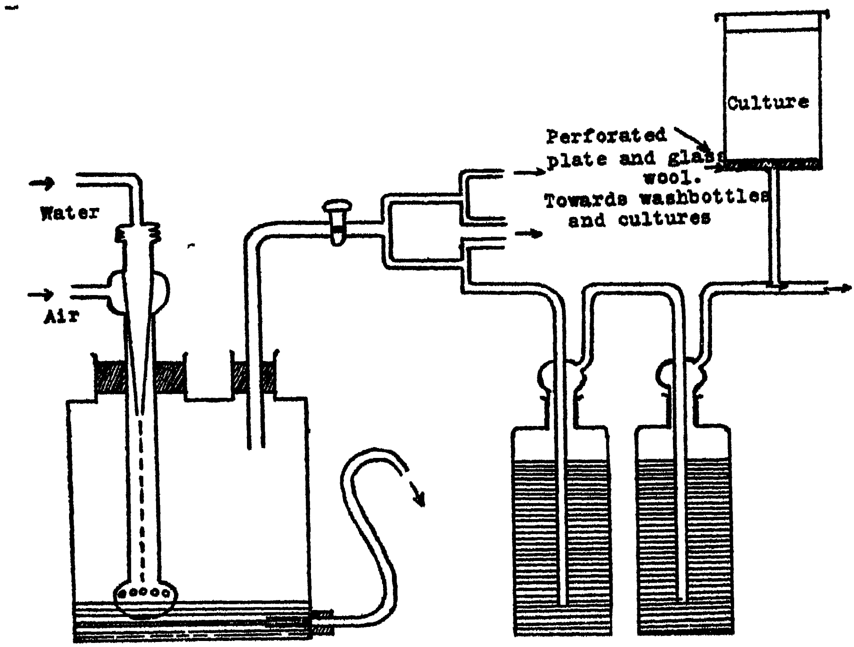

Water
Air
Culture
Perforated plate and glass wool.
Towards washbottles and cultures

FIG. 3. APPARATUS USED IN AERATION EXPERIMENTS

Washbottles contain water or sulfurous acid. Stream of air is regulated by stopcock

The stirred mixtures increased regularly in acidity, while the mixtures to which ammonium sulfate was added, in spite of the neutral calcareous soil, produced from the very beginning a much greater acidity than the check cultures without ammonium sulfate. The soluble P_2O_5 in these mixtures at

TABLE 11

Influence of aeration on the change of hydrogen-ion concentration, production of acidity and soluble phosphoric acid

NUMBER	TREATMENT	INITIAL REACTION		AFTER 6 WEEKS		AFTER 10 WEEKS		AFTER 12 WEEKS		AFTER 14 WEEKS		
				Reaction		Reaction		Reaction		Reaction		Soluble P_2O_5
		*cc.**	*pH*	*cc.*	*pH*	*cc.*	*pH*	*cc.*	*pH*	*cc.*	*pH*	*per cent*†
1	Stream of moist air	0.4	6.8	0.0	6.9	0.4	6.8	1.1	6.6	4.7	6.3	0.00
2	0.2 per cent $(NH_4)_2SO_4$ stream of moist air	0.4	6.8	11.2	5.1	10.8	5.6	16.5	5.3	9.5	5.5	trace
3	0.2 per cent $(NH_4)_2SO_4$, 10 cc. sulfurous acid added at start, stream of moist air	1.6	6.4	11.0	5.1	7.6	5.7	13.3	5.5	5.7	5.9	0.00
4	0.2 per cent $(NH_4)_2SO_4$, 10 cc. sulfurous acid in water through which air ran	1.6	6.4	71.0	3.6	58.1	3.7	62.1	4.2	37.0	5.0	trace
5	None	0.4	6.8	1.0	5.7	3.2	5.6	14.6	4.9	18.1	4.8	0.00
6	0.2 per cent $(NH_4)_2SO_4$	0.4	6.8	57.7	3.8	131.7	3.2	154.0	3.3	170.0	3.0	9.66
7	0.2 per cent $(NH_4)_2SO_4$, 10 cc. sulfurous acid added at start	1.6	6.4	57.4	3.7	141.7	3.1	165.9	3.3	180.5	3.0	10.70
8	0.2 per cent $(NH_4)_3SO_4$, 10 cc. sulfurous acid added by stirring	1.6	6.4	59.8	3.8	152.4	3.1	174.2	3.2	190.0	3.1	9.86

the end of 14 weeks was approximately 10 per cent, all being close together as well in acidity as in available phosphoric acid.

There seems no doubt but that in these experiments too great a quantity of air does not favor sulfur oxidation by the organisms as was supposed. There seems to be a limit to the quantity of oxygen which acts favorably on the organisms, but where the limit lies could not be determined. The quantities of air which went through, namely 24 liters per 500 gm. per day, were apparently too great, although 200 liters per ton per hour does not seem such an extreme abundance.

Observations

It was noticed throughout a number of series that the relative acidity increased regularly, but the intensity of the acid produced as measured by the hydrogen-ion concentration, varied. The concentrations became higher, till a certain point (3.1 to 2.9) was reached, then became slightly weaker and then back to the same point as before (3.1 to 2.9) to remain there. This point seemed to indicate when the greatest production of available phosphoric acid began. The hydrogen-ion concentration, however, does not necessarily have to be at a certain point, pH 3.2 for instance, to mark the beginning of the availability of phosphoric acid.

TABLE 12

Relative acidity, pH values and available phosphoric acid in aggregates as compared with the mixed compost

MATERIAL	REACTION		CITRATE SOLUBLE P_2O_5
	*cc.**	*pH*	*per cent*†
Coarse aggregates (about 4–5 mm. in diameter)	197.0	3.0	16.48
Medium size aggregates (about 2–3 mm. in diameter)	190.2	3.1	15.92
Small aggregates (about 1 mm. in diameter)	185.6	3.1	12.38
Finely sifted mixture	121.8	3.2	8.86
Unsifted mixture	171.6	3.1	11.42

* † Same as table 1.

The titration measurements of the relative acidity were affected by the soluble phosphates, etc., and they do not, therefore, express the exact amounts of sulfates and free acid formed. The constant action of the free acid on the tri-calcium phosphate may account for the irregularities noticed in the determinations of the hydrogen-ion concentrations. The point at which the greatest amounts of P_2O_5 are made available corresponds with the hydrogen-ion concentration of acid phosphate, which is generally 3.0 to 3.2 as expressed in pH values.

Throughout this work it was freuently noticed that the mixtures had a tendency to form small aggregates composed of soil, rock phosphate and sulfur. Upon examination of these aggregates under the microscope they appeared to have a peculiar structure of indefinite form. A great number of these aggregates were picked out and their relative acidity, hydrogen-ion concentration and available P_2O_5 determined. A part of the mixture as ordinarily taken was used for similar determinations, and another part cautiously sifted. The differences found are tabulated in table 12.

On account of this peculiarity these aggregates or crumbs were later on broken up when the composts were mixed and when samples were taken for analyses.

Often it was found that the incubated mixtures produced hydrogen sulfide during the first weeks of the incubation period. Sometimes it was only noticeable by the smell and at other times even by a black color produced. Small additions of sulfuric acid favored the sulfur-oxidizing organisms and depressed the hydrogen sulfide-producing organisms. Such cultures were always excluded in the final results and are not included in the tables.

CONCLUSIONS

1. Sulfur oxidation takes place in a calcareous slightly alkaline soil and does not hinder the solubility of phosphoric acid.

2. Compost mixtures incubated at 30°C. increased more rapidly in relative acidity and available P_2O_5 than composts kept at room temperature. When larger quantities of a calcareous soil is used the influence of temperature is usually greater than when small amounts of such a soil are employed.

3. The influence of light is slightly detrimental to sulfur oxidizing-organisms.

4*a*. Small quantities of sulfuric acid stimulate bacterial activities particularly during the first weeks of the incubation period, if calcareous, slightly alkaline soil is used.

b. Sodium bicarbonate stimulated the bacterial activities considerably when a calcareous soil poor in organic material was used; if a similar soil rich in organic material was used it failed to produce stimulation to the same extent, presumably due to greater CO_2 production in this soil.

c. Sodium bisulfate in solution and acetic acid had no stimulating effect, but rather detrimental.

d. A mixture of 0.02 per cent ferrous sulfate and 0.02 per cent aluminum sulfate failed to exert any influence.

5. Partial sterilization of the soil and the mixtures by additions of sodium chloride and by hydrochloric acid proved of no value. NaCl retarded the activities of the sulfur-oxidizing organisms, and the same was true to a less extent with HCl.

6*a*. The amounts of soil can be reduced with success from 16 to 17 per cent to 1.6 to 1.7 per cent of the mixtures.

b. It is possible to replace the bulk of a calcareous soil poor in nitrogenous material with ammonium sulfate.

7. The quantities of sulfur in the mixtures, reduced to approximately the amounts required in ordinary factory methods of making acid phosphate, gave good results.

8. Addition of small amounts of sulfurous acid to change the initial reaction of these mixtures in which a slightly alkaline calcareous soil was used, proved to have a decided influence upon the rapidity of accumulation of acidity and available phosphoric acid.

9. Aeration of the mixtures had considerable influence, but when the mixtures received an abundance of air, sulfur oxidation nearly ceased and no phosphoric acid was made available.

10. The hydrogen-ion concentration in the mixtures changed till pH values of from 3.1 to 2.9 were reached, indicating the point at which P_2O_5 becomes most rapidly available. Relative acidity, as measured by the titration method, accumulated after this point was reached.

11. The mixtures have a tendency to form aggregates which have a different relative acidity and different quantities of available P_2O_5 according to the size of the crumbs.

REFERENCES

(1) Brown, P. E., and Gwinn, A. R. 1917 Effect of sulfur and manure on the availability of rock phosphate in soil. Iowa Agr. Exp. Sta. Res. Bul. 43.

(2) Brown, P. E., and Kellogg, E. H. 1914 Sulfofication in soils. Iowa Agr. Exp. Sta. Res. Bul. 18.

(3) Clark, W. M., and Lubs, H. A. 1917 The colorimetric determination of hydrogen-ion concentration and its application in bacteriology. *In* Jour. Bact., v. 2, p. 1–34.

(4) Ellett, W. B., and Harris, W. G. 1920 Coöperative experiments for the composting of rock phosphate and sulfur. *In* Soil Sci., v. 10, p. 315–325.

(5) Gillespie, J. L. 1916 The reaction of soil and measurements of hydrogen-ion concentration. *In* Jour. Wash. Acad. Sci., v. 6, p. 7–16.

(6) Hilgard, E. W. 1906 Soils, Their Formation, Properties, Compositions and Relations to Climate, and Plant Growth in Humid and Arid Regions. MacMillan, New York.

(7) Hutchison, H. B. 1913 The partial sterilization of the soil by means of caustic lime. *In* Jour. Agr. Sci., v. 5, p. 1–36.

(8) Lindet and Bruno, A. 1918 Solubilité et assimilibilité des phosphates de chaux. *In* Compt. Rend. Acad. Agr. de France, v. 4, p. 692–694.

(9) Lipman, J. G., and Joffe, J. S. 1920 The influence of initial reaction on the oxidation of sulfur and the formation of available phosphates. *In* Soil Sci., v. 10, p. 327–332.

(10) Lipman, J. G., McLean, H. C., and Lint, C. H. 1916 The oxidation of sulfur in the soils as a means of increasing the availability of mineral phosphates. *In* Soil Sci., v. 1, p. 533–539.

(11) Lipman, J. G., McLean, H. C., and Lint, C. H. 1916 Sulfur oxidation and its effects on the availability of mineral phosphates. *In* Soil Sci., v. 2, p. 499–538.

(12) McLean, H. C. 1918 The oxidation of sulfur by microörganisms in its relation to the availability of phosphates. *In* Soil Sci., v. 5, p. 251–290.

(13) Rudolfs, W. 1922 Sulfur oxidation in "black alkali" soils. *In* Soil Sci., v. 13, p. 215–229.

(14) RUSSELL, E. J. 1917 The recent work at Rothamsted on the partial sterilization of soil. *In* Internat. Rev. Sci. and Pract. Agr., v. 8, p. 673–681.

(15) SHEDD, O. M. 1917 Effect of sulfur on different crops and soils. *In* Jour. Agr. Res., v. 11, p. 91–103.

(16) SHEDD, O. M. 1919 Effect of oxidation of sulfur in soils on the solubility of rock-phosphate and on nitrification. *In* Jour. Agr. Res., v. 18, p. 329–345.

(17) TRUFFAUT, G. 1918 Sur la stérilisation partielle du sol. *In* Compt. Rend. Acad. Agr. de France, v. 4, p. 1030–1038.

THE INFLUENCE OF SOIL REACTION UPON THE GROWTH OF ACTINOMYCETES CAUSING POTATO SCAB[1]

SELMAN A. WAKSMAN

New Jersey Agricultural Experiment Stations

Received for publication September 2, 1921

The close correlation between soil reaction and the growth of specific microörganisms in the soil has recently been made the subject of investigations from various viewpoints. By expressing the soil reaction in terms of hydrogen-ion concentrations, we are employing a factor which has greater significance in biochemical soil processes than merely the amount of acid substance present. In studying the relation between the hydrogen-ion concentration of the soil and the activities of microörganisms, we must differentiate between the limiting reaction, at which beneficial microörganisms become active in the soil, from that reaction at which microörganisms injurious in one way or another to the soil microflora or the growth of higher plants are checked in their development. The work of Gainey (7) and others takes up the influence of reactions upon the growth of *Azotobacter*, Fred and associates (6) and others on *B. radicicola*, Meyerhof (24) on Nitrosomonas and Nitrobacter. The limitation of the growth of actinomycetes causing potato scab can serve as an excellent illustration of the second group of phenomena. However, when we attempt to adjust the reaction of the soil to a certain hydrogen-ion concentration with a view of limiting the growth of an injurious organism, we ought not omit from consideration the influence that this reaction may exert upon the growth of beneficial organisms.

The organism causing potato scab was first described by Thaxter (28, 29) under the name *Oospora scabies*. In a study of "scab" of sugar beets, Krüger (17) suggested that Oospora can probably be classified with the streptothrices (actinomycetes). It was later identified by Cunningham (5) with *Actinomyces chromogenus* of Gasperini. However, the term *A. chromogenus* based upon the fact that the organism produces a dark brown pigment on organic media is hardly appropriate, since, as pointed out by Krainsky (16), Waksman (31, 32), and Conn (4), there is a large number of actinomycetes in the soil, which are able to produce this pigment. This factor was readily recognized by Güssow (12), who very appropriately suggested the term *Actinomyces scabies*, which should be used in the interests of an accurate nomenclature. Even with a correct nomenclature, we must be on the lookout for other actinomycetes not of the *A. scabies* type. From 15 to 40 per cent of the soil's microbial flora consists of actinomycetes and one may readily isolate from a diseased potato tuber another Actinomyces which will not be "scabies" at all and which may differ much in its metabolism, including the limiting reaction for growth. Only recently, Wollenweber (38) pointed out the potato scab is caused not by one species of Actinomyces, but by a number of them. At least 6–7 Actinomyces species have been isolated from diseased potatoes and from the soil, and their pathogenicity demonstrated by inoculation tests. As a result of these studies of Wollenweber and of the writer, which will be published at a later date, we may have to limit the term *A. scabies* (Thaxter) Güssow to one specific type, from which other actinomycetes that are able to cause potato scab may be differentiated. However, for the present, we may speak of the organism causing common potato scab both in America and in Europe (as pointed out by Wollenweber), as the *Actinomyces scabies* group.

---

[1] Paper No. 50 of the Journal Series, New Jersey Agricultural Experiment Stations, Department of Soil Chemistry and Bacteriology. This paper will appear in volume 1 of the RUTGERS COLLEGE STUDIES.

One of the most important and most promising methods of controlling the development of potato scab in the field is the use of acid forming substances. Fertilizers which leave the reaction of the soil alkaline have been looked upon as injurious, while those which leave the soil acid, like ammonium sulfate and acid phosphate, are generally preferred. The use of sulfur for combating scab has been recognized by Halsted (13, 14). But the results obtained by the various investigators are lacking in uniformity. Even Halsted (15) found that an application of 300 pounds of sulfur per acre was effective, within one year under certain conditions, while an application of 600 pounds of sulfur in 1896 was ineffective, but became effective in 1898.

These discrepancies were due to the fact that the basic underlying principles were not recognized, namely that the sulfur is oxidized in the soil by certain microörganisms to sulfuric acid, and the increase in soil acidity leads to a decrease in scab. Two factors are to be considered here; the presence of the proper sulfur-oxidizing organisms in the soil and the reaction and character of the soil itself. The amount of sulfur to be used will depend upon the latter factor.

This lack or correlation between the application of sulfur and control of scab is also true of the other experiments where the addition of sulfur failed to diminish the amount of scab as those of Garman (8) and Brooks (1). However, where enough sulfur was used year after year and the soil conditions were proper, a definite reduction of the potato scab was effected by Wheeler and Adams (36), Sherbakoff (26, 27) and Lint (18, 19). Even in as late a publication as that of Wollenweber (38) in 1920, where the importance of making the soil acid for the control of potato scab has been well recognized, the application of sulfur was not found to give uniform results.

Only with the development of methods for determining the correct reaction of the soil as expressed in terms of hydrogen-ion concentration, and with an increased knowledge of the microörganisms concerned in the oxidation of sulfur in the soil, could we understand the underlying principles and place our knowledge of scab control on a systematic foundation. A third factor should be considered here which is not of least importance, and that is the metabolism of the causative agent of potato scab, *A. scabies*. Our information on the soil reaction has been advanced by the researches of Sörensen and Michaelis in Europe, Clark and Lubs and others and particularly that of Gillespie (10, 11) in this country, as applied to scab control. Our knowledge of the mechanism of sulfur oxidation in the soil by crude cultures of microörganisms has been advanced by the work of Lipman and his associates (20, 23) and McLean (24), and with pure cultures by Waksman and Joffe (33, 34, 35). As to the metabolism of *Actinomyces scabies* itself, it has been taken up by Lutman and Cunningham (21) and by Waksman (30).

Considering the practical application of the subject, the following questions are important:

1. Is there a limiting acid reaction for the species of *Actinomyces scabies* which will absolutely prevent their development?
2. By what certain means can we obtain this reaction?

3. What is the influence of type of soil, amount of organic matter in the soil, moisture content, aeration, etc.?

These three points will be taken up in order and experimental results presented.

LIMITATION OF GROWTH OF A. SCABIES BY ACIDITY OF MEDIUM OR SOIL

Gillespie and Hurst (10) were the first to call attention to a characteristic difference of hydrogen-ion concentration shown by the extracts of soils of the types Caribou loam and Washburn loam. These types, similar in texture and derived from the same glacial till, differ, however, due to local topographic differences. In reaction, the Caribou loam is more intensely acid than the Washburn, with a relative freedom from scab of potatoes grown in the more acid soil. Gillespie and Hurst (11) have further shown that soils having a hydrogen-ion concentration equivalent to pH 5.2 rarely produced scabby potatoes, while less acid soils generally produced scabby potatoes, unless it was new land. In another paper, Gillespie (9) found that, by growing the organisms causing potato scab on two synthetic media and upon a medium prepared from potato extract at reactions of pH = 5.2 and less, the growth was much slower and generally less vigorous than in less acid media. It is interesting to note that Gillespie (9) observed a marked decrease in acidity accompanying the growth of the organisms. This change in reaction of media was pointed out by Waksman (31) for the Actinomyces group as a whole. Wollenweber (38) stated that actinomycetes causing potato scab are very sensitive to acid (0.05 per cent) and much less sensitive to alkali (0.25 per cent).

EXPERIMENTAL

The influence of reaction upon the growth of actinomycetes, in general, and species causing potato scab, in particular, was studied both in solution and in soil. The cultures used were obtained from Dr. Morse of the Maine Agricultural Experiment Station and Mr. M. Shapovalov of the U. S. Department of Agriculture except for three which were isolated by Waksman and Curtis (32).

The following cultures were used in one or another of the experiments:

A. scabies 222 and 254 were obtained from Dr. Morse, who isolated them from potatoes in Maine in 1906 and in 1914 respectively.

A. scabies 259 was obtained from Dr. Morse, who isolated if from potatoes coming from Ohio in 1915.

A. scabies 281, 283 and 295 were obtained from Mr. Shapovalov, who isolated them from potatoes infected with common scab in Maine.

A. griseus was isolated by the writer and Curtis (32) from the soil in 1916. This culture has a strong proteolytic power (30).

A. violaceus-ruber and *A. viridochromogenus* were also isolated in 1916 by the writer and Curtis from the soil and described in detail elsewhere (31). These two cultures are quite similar, in description, to Wollenweber's *A. tricolor* and *A. aerugineus*.

The organisms were grown on synthetic agar, Czapek's or dextrose agar (31), and were then inoculated into the sterile liquid medium. For the inoculation of sterile soil, the cultures were grown on the liquid synthetic media, in 250-cc. Erlenmeyer flasks and, for inoculation, 1 cc. of the culture, well shaken previously, was introduced, under sterile conditions, into each flask.

Experiment 1

The medium used in the first experiment had the following composition:

	grams
Glycerin	20.0
Asparagin	5.0
Mg SO_4	0.5
KCl	0.5
Fe SO_4	0.01

Phosphates in varying amounts. Distilled water to make 1000 cc. (after adding the buffers).

Phosphoric acid, KH_2PO_4, K_2HPO_4 and NaOH were used to adjust the reaction of the medium to the desired hydrogen-ion concentration, as shown in table 1.

TABLE 1

The amounts of buffer solutions used in experiment 1 and the hydrogen-ion concentration of the media

MEDIUM NUMBER	BUFFER SOLUTIONS				REACTION
	H_3PO_4 *1.0 N*	KH_2PO_4 *1.0 M*	K_2HPO_4 *1.0 M*	NaOH *1.0 N*	
	cc.	*cc.*	*cc.*	*cc.*	*pH*
1	4	16	....	....	3.2
2	2	18	....	....	3.6
3	....	20	....	....	4.2
4	....	19.5	0.5	....	4.6
5	....	19.0	1.0	....	5.2
6	....	18.0	2.0	....	5.6
7	....	16.0	4.0	....	6.4
8	....	4.0	16.0	....	7.0
9	....	....	20.0	....	7.5
10	....	....	16.0	4	8.2

In all media, a total of 20 cc. of the buffer solutions were added per liter. The media were distributed, in 100-cc. portions into 250-cc. Erlenmeyer flasks and sterilized at 15 pounds pressure for 15 minutes. The flasks were then inoculated from slants with a culture 7–14 days old by means of a platinum loop. They were incubated at 25–27°C. for 21 days and filtered through weighed filter paper. The growth on the filter paper was dried at 80°C. to constant weight. Under "growth", the relative amount of growth is given, while under "weight" actual weight of the mycelium is given, in milligrams. The pH values were obtained by the use of the indicator method, as outlined by Clark and Lubs (2). Results are presented in table 2.

The limiting acid reaction for the two cultures of *A. scabies* seems to be at a pH between 4.6 and 5.0, while the limiting acid reaction for *A. griseus* and *A. violaceus ruber* isolated from the soil lies between pH 4.2 and 4.6. This indicates a greater acid resistance for the saprophytic forms. As to the alkaline limit, *A. scabies* 259 did not develop above pH 7.4. When good growth took place, the reaction of the medium was invariably changed to alkaline. This is due to the fact that the actinomycetes as a group, with perhaps few exceptions, do not seem to form any acid from the carbon sources, as in the case of many bacteria and fungi, but produce alkaline substances from the nitrogen sources [Waksman (30)]. In this case, it is the production of ammonia from the asparagin which makes the medium alkaline. By determining the ammonia formed, we can account almost quantitatively for the change in reaction.

TABLE 2

Growth of actinomycetes in synthetic media at various hydrogen-ion concentrations and changes of the reaction produced.

MEDIUM	REACTION OF CONTROL	A. SCABIES 259			A. SCABIES 222			A. GRISEUS			A. VIOLACEUS RUBER		
		Relative growth*	Wt.	Final reaction	Relative growth	Wt.	Final reaction	Relative growth	Wt.	Final reaction	Relative growth	Wt.	Final reaction
	pH		*mgm.*	*pH*		*mgm.*	*pH*		*mgm.*	*pH*		*mgm.*	*pH*
1	3.4	0	0	3.4	0	0	3.4	0	0	3.4	0	0	3.4
2	3.8	0	0	3.8	0	0	3.8	0	0	3.8	0	0	3.8
3	4.2	0	0	4.2	0	0	4.2	0	0	4.2	0	0	4.2
4	4.6	0	0	4.6	0	0	4.6	T	1†	4.6	T	2†	4.6
5	5.0	T	1	5.0	2	268	5.0	T	1†	5.0	1	15	5.0
6	5.6	T	1	5.6	3	346	5.8	T	1†	5.6	2	47	5.8
7	6.4	1	2	6.4	4	377	6.8	2	214	6.4	3	184	6.6
8	6.9	2	5	7.1	5	601	8.4	3	253	8.0	4	314	7.4
9	7.4	1	2	7.4	5	499	8.6	2	177	8.2	3	233	7.6
10	8.0	0	0	8.0	5	547	9.2	2	110	9.0	1	99	8.0

* T = Trace, 1–5 indicates the relative amounts of growth, 5 being the maximum.

† Approximate weight.

Experiment 2

The medium used in this experiment had the same composition as that used in the previous one, only the amount of asparagin was reduced to 2 gm. per liter of medium and the amounts of buffers in the media were slightly changed, as shown in table 3. The cultures were grown for 21 days at 25–27°C. The results are given in table 4.

We find a decided difference between the various cultures of *A. scabies* in their resistance to acidity. While *A. scabies* 254 did not begin to develop till the pH of the medium was 5.3, *A. scabies* 281 made some growth even at 4.6.

TABLE 3

The amounts of buffer solutions used in experiment 2

MEDIUM NUMBER	BUFFER SOLUTION		REACTION OF MEDIUM
	KH_2PO_4 1 0 M	K_2HPO_4 1 0 M	
	cc.	*cc.*	*pH*
1	20.0	0.0	4.2
2	19.5	0.5	4.6
3	19.0	1.0	5.0
4	18.5	1.5	5.4
5	18.0	2.0	5.6
6	16 0	4.0	6.2
7	10.0	10 0	6.8

TABLE 4

Growth of A. scabies in synthetic media at various hydrogen-ion concentrations

MEDIUM NUMBER	REACTION OF CONTROL	A. SCABIES 254		A. SCABIES 259		A. SCABIES 281	
		Relative growth	Final reaction	Relative growth	Final reaction	Relative growth	Final reaction
	pH		*pH*		*pH*		*pH*
1	4.0	0	4.0	0	4.0	0	4.0
2	4.6	0	4.6	0	4.6	1	5.2
3	4.9	0	5.0	1	6.6	2	5.8
4	5.3	2	5.4	2	6.1	3	6.2
5	5.6	3	6.8	2	6.2	3	6.6
6	6.1	3	6 6	3	7.4	....	....
7	6.8	....	....	3	7.4	....	....

Experiment 3

The above experiment was repeated with larger amounts of buffer (50 cc. of the mixture) and 2 gm. of asparagin per liter. The flasks contained only 60 cc. of culture medium. Five cultures of *A. scabies* were used, with three or four flasks for each culture and each reaction. The amount of buffer used in experiment 3 is given in table 5 and the results are presented in table 6.

TABLE 5

The amount of buffer solutions used in experiment 3

MEDIUM NUMBER	KH_2PO_4 1 0 M	K_2HPO_4 1 0 M	REACTION OF MEDIUM
	cc.	*cc.*	*pH*
1	50.00	0.00	4.2
2	48.75	1.25	4.8
3	47.00	3.00	5.2
4	45.00	5.00	5.6
5	40.00	10.00	6.4

From the results presented in table 6, we may definitely establish the fact that, when the nutrients present in the medium are favorable for the development of actinomycetes, the limiting acid reaction, for the majority of strains, lies at a pH between 4.8 and 5.2. While some strains (281) may develop to a slight extent at 4.8 and, given a long enough period of incubation may even make a fair growth at that reaction, others (295) will begin to develop only at a much higher point, namely at about pH 5.6. It is quite possible that in the first case, the organism is able to change rapidly the reaction of the medium to less acid and make conditions more readily favorable for its de-

TABLE 6

Growth of A. scabies in synthetic media at various hydrogen-ion concentrations

INCUBATION PERIOD	REACTION OF MEDIUM	A. SCABIES 222			A. SCABIES 254			A. SCABIES 281			A. SCABIES 283		A. SCABIES 295	
		gr.*	wt.	pH	gr.	wt.	pH	gr.	wt.	pH	gr.	pH	gr.	pH
days	*pH*		*mgm.*			*mgm.*			*mgm.*					
	4.2	0			0			0			0		0	
	4.8	0			0			0			0		0	
5	5.2	1			0			0			1		0	
	5.6	2			T			0			2		0	
	6.4	3			2			1			3		1	
	4.2	0			0			0			0		0	
	4.8	0			0			0			0		0	
10	5.2	2			1			1			2		0	
	5.6	3			2			2			3		T	
	6.4	4			3			3			5		2	
	4.2	0			0			0			0		0	
	4.8	0			0			T			0		0	
15	5.2	3			1			2			2		0	
	5.6	4			2			3			4		1	
	6.4	5			3			4			5		2	
	4.2	0		4.2	0		4.2	0		4.2	0	4.2	0	4.2
	4.8	0		4.8	0		4.8	T	4	5.2	0	4.8	0	4.8
20	5.2	3	80	5.4	1	10	5.4	2	18	5.8	2	5.5	0	5.2
	5.6	5	190	6.5	3	60	6.3	4	42	6.7	4	6.2	1	5.9
	6.4	5	225	6.9	3	48	6.8	4	120	7.1	5	7.0	2	6.6
	4.2	0		4.2	0		4.2	0		4.2	0	4.2	0	4.2
	4.8	0		4.8	0		4.8	2	30	6.3	T	5.0	0	4.8
30	5.2	3	70	5.4	2	40	5.8	3	60	7.3	3	5.4	0	5.2
	5.6	5	200	7.1	4	120	6.6	3	65	6.8	5	6.4	1	6.0
	6.4	5	250	7.3	4	125	6.8	4	100	7.4	5	7.0	2	6.6

* gr. = relative growth; wt. = actual weight of mycelium; pH = pH value of culture at the end of the particular period.

velopment.

velopment than do the other strains. Culture 295 made a rather weak growth even under optimum conditions; this is due probably to the fact that this strain has lost its power of producing aerial mycelium, perhaps on account of long cultivation on artificial media. When a piece of substratum growth is inoculated into a flask with medium, conditions have to be very favorable for its development, before growth can take place. Aerial spores, however, soon germinate and develop into a mycelium, if conditions are only favorable. This is the reason why so many variable results are obtained with various cultures. It is due more to the length of time the organism has been kept on artificial culture media than to a difference in strain. Wollenweber (38) has obtained such a culture from Amsterdam, this culture coming originally from Dr. Whetzel's laboratory at Cornell University. Unable to obtain any scab on potatoes by the use of this culture, Wollenweber merely suggested that it is possible that the American strain does not cause potato scab in Germany.

The change in reaction of medium to less acid confirms the previous observations.

Experiment 4

In addition to the study of the growth of actinomycetes in solution, a series of experiments were carried out, in which actinomycetes, chiefly *A. scabies*, were grown in sterile soil previously adjusted to various reactions, with or without the addition of organic matter. A greenhouse soil and a Sassafras loam were used for this work. A quantity of the two soils were air dried, sieved and brought into the laboratory. The total moisture holding capacity of these soils was 60 and 30 per cent respectively. During the experiment the soils were kept at 60 to 70 per cent saturation unless otherwise stated. The soils, with or without the organic matter, were placed, in 100-gm. portions, in 250-cc. Erlenmeyer flasks, moisture was added, and flasks were plugged with cotton and sterilized in the autoclave at 15 pounds pressure for 1 hour. The flasks were then inoculated with 1-cc. portions of well shaken liquid cultures of the proper organisms, 7 to 14 days old, and incubated at 25 to 28° for the desired length of time. Sterile water was added, at definite intervals, to keep the moisture at an optimum. At the end of the incubation period, some of the soil was used for determinations of ammonia. The method of Gillespie (10) for obtaining the soil solution combined with the colorimetric method for the determination of reaction were used. Ammonia was determined by distillation with MgO into standard acid solution. In some cases the Folin aeration method was used; the results checked up fairly well with those obtained by the distillation method, but were somewhat lower. Since the ammonia formation is given only as an indication of the growth of the organisms and is not an absolute value only the results obtained from the distillation with MgO are reported.

In the first experiment, the greenhouse soil was used; the reaction was adjusted to various pH values by means of sulfuric acid and CaO. Ten-gram portions of soil were placed in beakers, and various amounts of acid and CaO added. To each soil 3 cc. of water was added (the acid was added in the water to make 3 cc.), the beakers covered and allowed to stand 4 to 7 days, when the pH values were determined. Titration curves for the two soils are given in figure 1.

Ten points were selected on the curve, so as to give a series of values, ranging from pH 3.0 to pH 9.8. The proper amounts of acid and CaO were then added to 1-kgm. portions of the soil, the optimum amount of water was then introduced and the soils allowed to stand in jars till air-dry. The soils were then placed in 100-gm. portions, in flasks, 2 gm. of alfalfa meal or 1 gm. of dried blood were then added, the soils were brought to optimum moisture, sterilized as usual, inoculated with *A. scabies* 222 and the three soil Actinomyces and incubated for 28 days. The flasks were well shaken at the end of 7 days, so as to make the inoculation more uniform. The results are given in table 7.

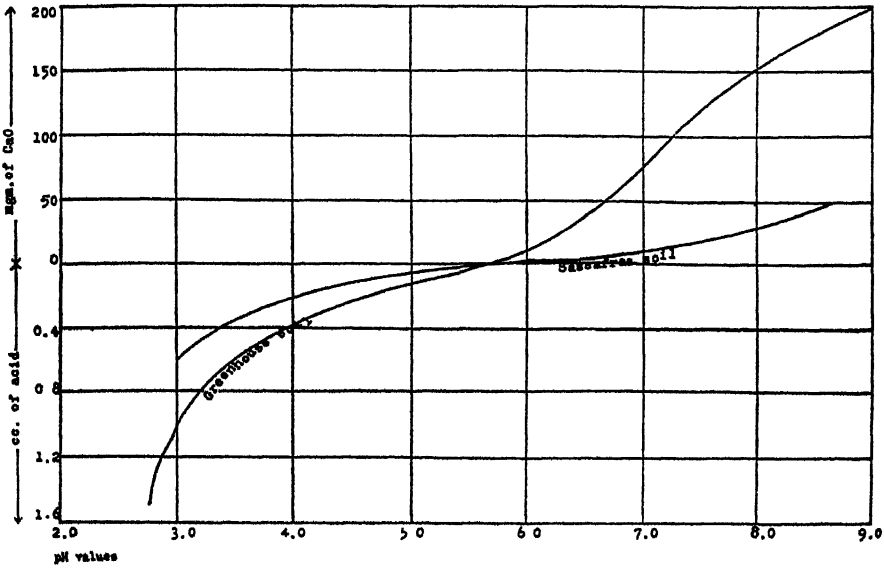


FIG. 1. TITRATION CURVES FOR THE TWO SOILS

The hydrogen-ion exponent (pH value) are given as ordinates and the amounts of 2 *N* acid, in cubic centimeters, or powdered CaO, in milligrams, added to 10-gm. portions of soil, as abscissae.

The sterilization influenced to some extent the reaction of the soil, particularly in the case of the soils made alkaline. In these the reaction became less alkaline, probably because of the absorption of the calcium. The limiting acid reaction is found to lie at about pH 5.0 to 5.2. While *A. scabies* produced,

TABLE 7

The influence of reaction upon the growth of actinomycetes in greenhouse soil

ORIGINAL REACTION OF MEDIUM	CONTROL			A. SCABIES 222						A. VIRIDOCHROMOGENUS						A. GRISEUS					
	Reaction		NH$_3$ in 100 gm. soil and dried blood	Afalfa		Dried blood				Alfalfa		Dried blood				Alfalfa		Dried blood			
								NH$_3$						NH$_3$						NH$_3$	
	Alfalfa	Dried blood		Gr.*	Final reaction	Gr.	Final reaction	In 100 gm. soil	Increase over contol	Gr.	Final reaction	Gr.	Final reaction	In 100 gm. soil	Increase over control	Gr.	Final reaction	Gr.	Final reaction	In 100 gm. soil	Increase over control
pH	*pH*	*pH*	*mgm.*		*pH*		*pH*	*mgm.*			*pH*		*pH*				*pH*		*pH*		
3.0	3.2	3.2	9.6	0	3.2	0	3.2	8.7	— 0.9	0	3 2	0	3.2	9.1	−0.5	0	3.2	0	3.2	9.3	— 0.3
3.6	3.6	3.6	10.7	0	3.6	0	3.6	9.8	— 0.7	0	3.6	0	3.6	13.7	3.1	0	3.6	0	3.6	11.6	1.0
4.2	4.1	4.0	10.7	0	4.2	0	4.5	12.9	2.2	0	4 2	0	4.4	11.6	0.9	0	4.4	0	4.3	11.8	1.1
5.2	5.0	5.0	9.9	T	5.2	2	6.0	38.8	28.9	T	5.2	2	6.0	19.3	9.4	T	5.2	1	6.2	11.2	1.3
5.8	5.8	5.8	9.1	2	6.0	3	7.3	77.4	68.3	2	6.0	3	6.6	56.6	47.5	1	6.2	2	7.0	50.0	40.9
6.6	6.4	6.4	5.8	3	6.6	3	8.0	70.1	64.3	3	6.6	4	8.0	71.0	65.2	3	7.3	3	8.0	65.8	60.0
7.0	6.8	7.2	5.2	3	7.6	3	8.1	72.1	66.9	4	7.7	3	8.4	68.5	63.3	2	7.8	3	8.4	67.2	62.0
7.7	7.7	7.7	4.6	2	8.0	3	8.4	67.2	62.6	3	8.0	2	8.4	68.2	63.6	2	8.3	3	8.6	57.6	53.0
9.4	8.8	8.8	3.2	T	9.0	T	9.4	3.6	0.4	1	9.0	0–1	8.8			1	8.6	2	8.8	~~6.0~~	2.8
9.8	9.4	9.6	2.4	0	9.6	0	9.6	2.1	— 0.3	0	9.6	0	9.6	2.4	0	0	9.8	0	9.8	2.8	0.4

* Gr. = relative growth.

at this reaction, only a trace of growth with the alfalfa meal, it made a fair growth with the dried blood. This is no doubt due to the rapid decomposition of the latter, with the formation of ammonia which accounts for the change in reaction. As much as fifty per cent of the nitrogen of the dried blood has been liberated in 28 days in the form of ammonia by various actinomycetes when the initial reaction was at an optimum. Although the figures given under growth are only relative, the amounts of ammonia formed are good indications of actual growth that has taken place. Under these conditions, we find that the optimum reaction for both *A. scabies* and the saprophytic actinomycetes is at a pH 5.8 to 7.7, while the limiting reactions are pH 5.0, on the acid side, and pH 8.8, on the alkaline. Above and below these two points no growth took place.

The alkaline limit is so high that it would be impossible to obtain it for any practical purposes, without ruining the fertility of the soil and is comparable only to black alkali soils. The acid limit is that found by Gillespie and Hurst (10, 11) for the potato soils of Maine which give good crops of potatoes practically free from scab. The application of this limit is not only possible, but practical and of immense economic importance.

Experiment 5

To eliminate the rapid change in reaction, due to the presence of easily decomposable organic matter, the experiment 4 was repeated with sassafras soil to which no organic matter had been added. Five strains of *A. scabies* were used. Since the amounts of ammonia formed from the soil organic matter were rather small, the actual numbers of actinomycetes were determined in the flask by the plate method. Of course, we do not get here absolute figures, since no differentiation is made between actinomycetes spores and mycelium. The results are, however, striking even with the relative figures. The method of plating out is similar to that used in the routine soil bacterilogical counts, modified egg albumen agar being used for preparing the plates. The details of the method are found elsewhere (31). The results are reported in table 8.

The differences found in the liquid cultures are also observed on sterile soil. *A. scabies* 281 makes a fair growth at pH 5.1, while 295 begins to develop only at pH 5.3. The rapidity of formation of aerial mycelium accounts for the difference in numbers. Cultures 222 and 283 readily form an abundant aerial mycelium and gave, therefore, a count of 80 millions per gram, with an optimum reaction. Culture 295, which formed no aerial mycelium in pure culture, grew much more slowly and gave a maximum of only 1,500,000 per gram. The limiting reaction for the majority of the strains is found at pH 5.0. Culture 281, however, grew at pH 4.8 and others developed only when the reaction was distinctly less acid, pH 5.3 to 5.5. The difference in the strains may also account for some of the discrepancies in scab control by making the soil acid.

TABLE 8

The influence of reaction upon the growth of A. scabies in the soil

ORIGINAL REACTION OF MEDIUM	A. SCABIES 222			A. SCABIES 254			A. SCABIES 281			A. SCABIES 283			A. SCABIES 295		
	Gr.*	Numbers†	Final reaction	Gr.	Numbers	Final reaction	Gr.	Numbers	Final reaction	Gr.	Numbers	Final reaction	Gr.	Numbers	Final reaction
pH			*pH*			*pH*			*pH*			*pH*			*pH*
4.8	0	15,000	4.8	0	8,000	4.8	0	7,000	4.9	0	110,000	4.9	0	3,000	4.9
5.1	1	800,000	5.3	0	9,000	5.1	1	48,000	5.4	?	330,000	5.2	0	20,000	5.1
5.3	2	1,700,000	5.6	1	500,000	5.4	2	500,000	5.6	1	22,000,000	5.5	T	36,000	5.3
5.6	3	51,000,000	5.8	3	1,000,000	6.0	3	1,700,000	5.6	3	40,000,000	5.7	1	250,000	5.5
6.2	4	80,000,000	6.2	3	1,500,000	6.3	3	2,500,000	6.2	3	47,000,000	6.4	2	300,000	6.0
7.2	4	85,000,000	7.4	4	5,000,000	7.6	3	10,300,000	7.7	4	80,000,000	7.5	2	1,500,000	7.6

* gr. = relative amount of growth.

† Organisms per gram of soil.

THE REDUCTION OF SOIL REACTION BY THE UTILIZATION OF THE OXIDATION OF SULFUR BY MICROÖRGANISMS

The use of acidic fertilizers, or such that tend to make the soil reaction more acid has been recommended. These may do for soils that are acid to start with and where the amount of scab may be small. In this case the use of green manures may suffice to control scab, because organic acids formed in the process of the decomposition of the organic matter may be sufficient to exert a temporary check upon the development of scab. However, this acidity is not of a lasting nature, since the organic acids are rapidly broken down in the soil.

To be able to control scab in slightly alkaline, neutral or only slightly acid soils, acid has to be added to the soil so as to make the reaction acid enough to prevent growth of *A. scabies*. The direct use of acid is both unpractical in method of application and injurious in its action upon the beneficial microorganisms. A substance has to be added which gradually becomes acid. Such a substance is sulfur, which will be oxidized to sulfuric acid, provided the proper sulfur oxidizing organisms are present.

A brief review of the literature on the use of sulfur for the control of potato scab has been given above. We might cite here two typical experiments which have a direct bearing upon the problem at hand.

Wheeler, Hartwell and Moore (37) found that by applying sulfur at the rate of 600 pounds per acre in 1896, no benefit was obtained in limiting scab, in several instances, although it seemed to have been of some value in other cases. When 300 pounds of sulfur per acre were applied again to the same soil in 1897, the number of scabbed tubers was reduced 9 per cent and the number of badly scabbed ones, 31 per cent. They suggested then, quite correctly, that sulfur is oxidized in the soil to sulfuric acid and the germicidal action of the sulfur was probably caused by this acid; therefore, a given amount of sulfur would be expected to exert a more marked influence as a preventative of scab on a netural or slightly alkaline soil than on one which was very alkaline or which contained such large quantities of carbonates as not to be materially affected by the limited amount of acid produced. However, they pointed out that the application of sulfur to soils, for preventing potato scab regardless of the character of the reaction of the soil is liable to occasionally cause much injury.

By applying sulfur to soils and studying the resulting reaction Martin (22) found that an increase in soil acidity resulting from oxidation of sulfur led to a marked decrease in the amount of scabby potatoes. Where the initial hydrogen-ion concentration of the soil before the sulfur application was pH 5.8 or less, the lighter applications 300 to 500 pounds of sulfur gave approximately as good control of scab as the heavier applications (700 to 1200 pounds); where the initial reaction of the soil was greater than pH 6.0, the heavier applications of sulfur gave the best control of scab.

Experiment 6

In a study of the influence of oxidation of sulfur of the soil upon the activities of the various groups of soil microörganisms, it was found that small additions of sulfur, as long as the reaction does not become too acid (not below pH 5.0), stimulate the growth of microörganisms as indicated by numbers and the decomposition of organic matter. Of course such microörganisms, like the nitrifying and nitrogen fixing bacteria, which are active at an optimum reaction above pH 6.0 and more, may be slightly injured in their activities (this has not been demonstrated yet). But as soon as the reaction becomes acid enough to bring down the pH value to less than 5.0 or 5.2, the relative number of actinomycetes to the total bacterial numbers rapidly decreases, as indicated by the results in table 9, which represents a typical instance.

TABLE 9

The influence of sulfur oxidation upon the relative numbers of bacteria and actinomycetes in the soil

SULFUR APPLIED PER ACRE	REACTION	MICROÖRGANISMS PER GRAM	ACTINOMYCETES	
			Numbers per gram	Per cent of total numbers
lbs.	*pH*			
None	6.2	13,600,000	6,100,000	44.8
300	5.6	12,600,000	6,500,000	51.6
600	5.1	4,800,000	1,200,000	25.0
900	4.8	4,000,000	900,000	22.5

Experiment 7

Several experiments were carried on for the purpose of demonstrating the fact that the oxidation of sulfur by a pure culture of *Thiobacillus thiooxidans* will result in preventing or limiting the growth of pure cultures of *A. scabies* in the same soil. But experiments of this nature are rather difficult to carry out. When sulfur and *Th. thiooxidans* are added first to the soil, and the *A. scabies* is introduced only after the oxidation of the sulfur has gone so far as to make the reaction of the soil acid enough, no growth of *A. scabies* will take place. This treatment is equivalent to making the soil acid by means of sulfuric acid. When *A. scabies* is introduced in the beginning of the experiment, it will make the reaction of the soil alkaline enough, particularly in the presence of a protein-rich substance like dried blood, to neutralize the acidity formed from the oxidation of sulfur. This is particularly true of sterile soil, where the actinomycetes begin to develop rather rapidly while the action of *Th. thiooxidans* is slow at first. These experiments are therefore not representative of what actually takes place in normal soil.

An experiment will therefore be reported, in which the greenhouse soil was used, both with and without the addition of 1 per cent dried blood. Where sulfur was used (100–150 mgm.), it was added to 100-gm. portions of the soil, before sterilization, which took place as usual. The relative amount of growth, ammonia content, and the numbers of actinomycetes per gram of soil are reported as an index of the activities of *A. scabies*. The flasks were inoculated with 1-cc. portions of a 14-day old liquid culture of *Th. thiooxidans* and *A. scabies* strains.

Whenever sulfur was used in connection with *Thiobacillus thiooxidans*, there was found a limitation of the growth of the various strains of *A. scabies*, as indicated by numbers and ammonia formation. However, the pH values are not those that we would expect from the previous experiments. We would expect that the reaction, in which the limitation of the growth of *A.*

TABLE 10

The influence of sulfur oxidation by Thiobacillus thiooxidans upon the development of A. scabies

TH. THIOOXIDANS	A. SCABIES STRAIN NO.	RELATIVE GROWTH	FINAL REACTION	NH_3 IN 100 GM. SOIL	ACTINOMYCETES PRESENT IN 1 GM. OF SOIL
Soil itself No sulfur					
			pH	*mgm.*	
+*		0	5.6	7.3	0
+	254	1	5.9	7.5	660,000
+	257	2	6.0	13.6	13,000,000
+	259	2	6.0	9.7	14,500,000
—	259	2	6.0	14.7	17,000,000
0.1 per cent sulfur					
+		0	2.8	7.1	0
+	254	T	5.8	7.1	18,000
+	257	T	5.8	9.5	0
+	259	T	5.8	7.5	500,000
—	259	1	5.8	10.3	3,000,000
Soil with 1 per cent dried blood No sulfur					
+		0	5.6	8.7	0
+	254	3	6.7	42.0	22,000,000
—	254	3	7.2	65.8	40,000,000
+	257	3	7.5	79.5	400,000,000
—	257	3	7.5	89.6	100,000,000
+	259	3	7.3	82.0	100,000,000
—	259	3	7.5	82.6	124,000,000
0.15 per cent sulfur					
+		0	5.6	8.6	0
+	254	T	5.8	12.8	275,000
—	254	1	6.0	23.2	9,000,000
+	257	0	5.6	8.0	0
—	257	3	6.4	32.3	17,000,000
+	259	2	5.8	39.0	5,000,000
—	259	3	6.3	56.0	130,000,000

* + indicates flasks inoculated with sulfur oxidizing organism; —, uninoculated.

scabies took place, should be at a pH of 5.0 or less. We find flasks, however, in which no growth of *A. scabies* took place, to have a pH value of 5.6 and even 5.8. The only explanation for that phenomenon is the very complexity of the problem and the fact that there was present such a large amount of organic matter. Possibly the sulfuric acid formed from the oxidation of sulfur was present on the surface of the soil particles, sufficient to exert a preventative action upon the germination and growth of *A. scabies*. When the soil is shaken up with water, for the pH determination, the acid may be rapidly neutralized by the buffers present in the soil, in the form of proteins and inorganic colloids. We will be able to answer this complicated problem more definitely when our information of the relation between the acid formation and surface phenomena of the soil as well as the diffusion of the acid will be more extended.

The important conclusion to be drawn from this experiment is the fact that the presence of sufficient sulfur with the sulfur oxidizing bacterium can prevent the development of Actinomyces species that cause potato scab.

INFLUENCE OF SOIL TYPE, MOISTURE, ETC., UPON THE DEVELOPMENT OF ACTINOMYCES SCABIES

According to Wollenweber (38), scab is not abundant in heavy loam and clay soils and humus-rich moor soils. Light sandy soils, especially in dry years are inclined very favorably to scabbiness. Millard (25) called attention to the fact that peat soils are usually free from scab, while in sandy or gravelly soils, especially where these have been liberally supplied with lime, the disease is prevalent. He explains this by the fact that the introduction of fresh organic matter remedies this defect since it serves as a food (acting as a decoy for the fungus), thus the potato escapes the attack.

It would seem to be rather contradictory to observations of those who studied the activities of actinomycetes in the soil. Conn (3), Waksman (31), Krainsky (16) found them to be quite abundant in soils rich in organic matter. Of course, the observation of Millard (25) that the organic matter serves as a sort of decoy for the *A. scabies* is untenable, both from the principles underlying activities of microörganisms and from available data.

The very accurate studies of Gillespie and Hurst (10) on the Caribou and Washburn soil types in Maine would seem to point to just the opposite results from the observations of the other investigators. The Washburn loam which, due to its low situation, favors an accumulation of muck and peat, is much more subjected to infection by *A. scabies*. Gillespie and Hurst pointed out quite accurately that the reasons for this difference are to be looked for in the difference in soil reaction as measured by the hydrogen-ion concentration. No attempt in this direction has been made by either the German (38) or the English (25) investigators.

We must also keep in mind that, in the presence of an abundance of organic matter, there is an abundant development of fungi and acid producing bacteria. These may produce enough acid from the decomposition of the organic matter to limit the growth of *A. scabies*. As a matter of fact, in slightly acid soils, a small amount of scab may be controlled by the use of green manure, which is rapidly decomposed by various soil microörganisms with the formation (temporary probably) of sufficient organic acids to control scab.

As to the influence of moisture and soil type, a brief summary of experiments carried on for that purpose will suffice here.

The optimum condition for the growth of various strains of *A. scabies* is found at a moisture content of about 60 to 65 per cent of the maximum moisture holding capacity of the soil. This optimum is not much different from that commonly employed as the optimum for the activities of soil bacteria and fungi.

When various amounts of sand were added to a medium loamy soil, the best growth of *A. scabies* was obtained with the mixture containing 25 per cent of sand. Upon a further addition of sand, growth rapidly decreased, till, in pure sand, the amount of growth (as indicated by the formation of ammonia from dried blood) was less than one-fourth that in the soil itself.

SUMMARY

1. The limiting acid reaction for the growth of *Actinomyces scabies* in culture solutions, properly buffered, and in soil, varies with the strain. For the majority of strains, the limiting acid reaction is about pH 5.0–5.2; some strains may grow even at pH 4.8, while others will begin to develop only at pH 5.3–5.6. These results bear out, in the main, the results of Gillespie.
2. The saprophytic soil actinomycetes seem to be more acid resistant than *A. scabies* strains.
3. By the use of the proper amount of sulfur inoculated with *Thiobacillus thiooxidans*, an acid reaction is obtained which will control the growth of *A. scabies* in the soil.

ACKNOWLEDGMENTS

The author is indebted to Dr. M. T. Cook of the New Jersey Agriculture Experiment Stations for reading the manuscript and to Mr. J. S. Joffe for assistance in some of the analytical work.

REFERENCES

(1) Brooks, W. P. 1897 Scab of potatoes. In Mass. (Hatch) Agr. Exp. Sta. 9th Ann. Rpt., p. 44–45.

(2) Clark, W. M., and Lubs, H. A. 1917 The colorimetric determination of hydrogen-ion concentration and its application to bacteriology. *In* Jour. Bact., v. 2, no. 1, p. 1–34, 109–136, v. 2, no. 3, p. 191–235.

(3) Conn, H. J. 1916 A possible function of actinomycetes in soil. N. Y. (Geneva) Agr. Exp. Sta. Tech. Bull. 52.

(4) Conn, H. J. 1917 Soil flora studies. V. Actinomycetes in soil. N. Y. (Geneva) Agr. Exp. Sta. Tech. Bul. 60.

(5) Cunningham, G. C. 1912 The relationship of *Oospora scabies* to the higher bacteria. *In* Phytopathology, v. 2, p. 97.

(6) Fred, E. B., and Davenport, A. 1918 Influence of reaction on nitrogen-assimilating bacteria. *In* Jour. Agr. Res., v. 14, p. 317.

(7) Gainey, P. L. 1918 Soil reaction and the growth of Azotobacter. *In* Jour. Agr. Res., v. 14, no. 7, p. 265–271.

(8) GARMAN, H. 1898 Corrosive sublimate and flour of sulfur for potato scab; experiments made in 1896. Ky. Agr. Exp. Sta. Bul. 72, p. 9–18.

(9) GILLESPIE, L. J. 1918 The growth of the potato scab organism at various hydrogen-ion concentrations as related to the comparative freedom of acid soils from the potato scab. *In* Phytopathology, v. 8, no. 6, p. 257–269.

(10) GILLESPIE, L. J., AND HURST, L. A. 1917 Hydrogen-ion concentration measurements of soils of two types; Caribou loam and Washburn loam. *In* Soil Sci., v. 4, no. 4, p. 313–319.

(11) GILLESPIE, L, J., AND HURST, L. A. 1918 Hydrogen-ion concentration—soil type—common potato scab. *In* Soil Sci., v. 6, no. 3, p. 219–236.

(12) GÜSSOW, H. T. 1914 The systematic position of the organism of the common potato scab. *In* Science, n. s., v. 39, p. 431–3.

(13) HALSTED, B. D. 1895 Field experiments with potatoes. N. J. Agr. Exp. Sta. Bul. 112.

(14) HALSTED, B. D. 1897 Field experiments with potatoes. N. J. Agr. Exp. Sta. Bul. 120.

(15) HALSTED, B. D. 1896–1899 Experiments with potatoes. *In* N. J. Agr. Exp. Sta. 17th Ann. Rpt., (1896) p. 309–328; 18th Ann. Rpt. (1897), p. 276–284; 19th Ann. Rpt. (1898), p. 299–309; 20th Ann. Rpt. (1899), p. 326–345.

(16) KRAINSKY, A. 1914 Die Aktinomyceten und ihre Bedeutung in der Natur. *In* Centbl. Bakt. (etc.), Abt. 2, 41, p. 639–688.

(17) KRÜGER, F. 1905 Untersuchungen über den Gürtelschorf der Zuckerüben. *In* Arb. aus Biol. Anstalt Land. Forstwirt. Berlin, v. 4, p. 254–318.

(18) LINT, H. C. 1914–1916 Report of potato scab experiments. *In* N. J. Agr. Exp. Sta. 35th Ann. Rpt. (1914), p. 477–488; 36th Ann. Rpt. (1915), p. 375–381; 37th Ann. Rpt. (1916), p. 618–625.

(19) LINT, H. C. 1914 The influence of sulfur on soil acidity. *In* Jour. Indus. Engin. Chem., v. 6, p. 747.

(20) LIPMAN, J. G., McLEAN, H. C., AND LINT, H. C. 1916 Sulfur oxidation in soils and its effect on the availability of mineral phosphates. *In* Soil Sci., v. 2, no. 6, p. 499–538.

(21) LUTMAN, B. F., AND CUNNINGHAM, G. C. 1914 Potato scab. Vt. Agr. Exp. Sta. Bul. 184.

(22) MARTIN, W. H. 1920 The relation of sulfur to soil acidity and to the control of potato scab. *In* Soil Sci., v. 9, no. 6, p. 393–408.

(23) McLEAN, H. C. 1918 The oxidation of sulfur by microörganisms in its relation to the availability of phosphates. *In* Soil Sci., v. 5, no. 4, p. 251–290.

(24) MEYERHOF, O. 1916 Untersuchungen über den Atmungsvorgang nitrifizierender Bakterien. *In* Arch. Physiol. (Pflüger), v. 164, p. 353.

(25) MILLARD, W. A. 1921 Common scab of potatoes. *In* Jour. Ministry of Agr. (London), v. 27, no. 1, p. 49–53.

(26) SHERBAKOFF, C. D. 1914 Potato scab and sulfur disinfection. N. Y. (Cornell) Agr. Exp. Sta. Bul. 350, p. 709–743.

(27) SHERBAKOFF, C. D. 1915 The after effect of sulfur treatment on soil. *In* Phytopathology, v. 5, no. 4, p. 219–222.

(28) THAXTER, R. 1890 The potato "scab." Conn. (State) Agr. Exp. Sta. Bul. 105.

(29) THAXTER, R. 1890–1891 The potato "scab". *In* Conn. (State) Agr. Exp. Sta. Ann. Rpt. (1890), p. 81–95; (1891), p. 153–160.

(30) WAKSMAN, S. A. 1919 Studies in the metabolism of actinomycetes. *In* Jour. Bact., v. 4, p. 189–216, 307–330.

(31) WAKSMAN, S. A. 1919 Cultural studies of species of actinomycetes. *In* Soil Sci., v. 8, no. 2, p. 71–215.

(32) WAKSMAN, S. A., AND CURTIS, R. E. 1916 The actinomyces of the soil. *In* Soil Sci., v. 1, p. 99–134.

(33) WAKSMAN, S. A., AND JOFFE, J. S. 1921 The oxidation of sulfur by microörganisms. *In* Proc. Soc. Exp. Biol. Med., v. 18, p. 1–3.

(34) WAKSMAN, S. A., AND JOFFE, J. S. Acid production by a new sulfur-oxidizing bacterium. *In* Science, n. s., v. 53, no. 1366, p. 216.

(35) WAKSMAN, S. A., AND JOFFE, J. S. 1922 *Thiobacillus thiooxidans*, a new sulfur oxidizing organism isolated from the soil. *In* Jour. Bact., v. 7, p. 239–256.

(36) WHEELER, II. J., AND ADAMS, G. E. 1897 On the use of flowers of sulfur and sulfate of ammonia as preventive of the potato scab in contaminated soils. R. I. Agr. Exp. Sta. 10th Ann. Rpt. p. 254–268.

(37) WHEELER, H. J., HARTWELL, B. L., AND MOORE, N. L. C. 1899 Upon the after effect of sulfur, when applied to soils for the purpose of preventing potato scab. R. I. Agr. Exp. Sta. Rpt. 12, p. 163–167.

(38) WOLLENWEBER, H. W. 1920 Der Kartoffelschorf. Arb. d. Forschungsinstitutes f. Kartoffelbau. Heft 2, Paul Parey, Berlin.

MICROBIOLOGICAL ANALYSIS OF SOIL AS AN INDEX OF SOIL FERTILITY: I. THE MATHEMATICAL INTERPRETATION OF NUMBERS OF MICROÖRGANISMS IN THE SOIL[1]

SELMAN A. WAKSMAN

New Jersey Agricultural Experiment Stations

Received for publication September 19, 1921

INTRODUCTORY

In 1909, Fischer (8) published a paper in which he came to the conclusion that we do not possess as yet a proper method for a bacteriological analysis of the soil, and that we have no proper information as yet for the interpretation of the fertility of the soil from a bacteriological point of view. Were we to consider that same question today, we should have to come to the same conclusion. All attempts to interpret crop production from a microbiological point of view have not been rich in consequences and some investigators, who have considered a few of the methods carefully, such as bacterial numbers, ammonification, denitrification, etc., have come to the conclusion that they are practically worthless for the interpretation of soil fertility. The only exception to this statement may be found in some of the recent investigations on nitrification.

This lack of definite information and the unreliability of the information that we do possess is due to a number of factors, chief among which is the lack of standard methods employed. Not only do the methods used at present in soil microbiological research vary in the various laboratories and different importance is attached to them, but samples taken from the soil and treated by the same methods in the same laboratory are often found to give varying results. This may be due to a number of factors: 1. The methods employed in the various laboratories are not standardized. 2. The work is usually carried out with very few unrepresentative samples taken from a small area. 3. The soil is considered as constant and usually no allowance is made for the variations between the different samples. 4. The small amount of exact information that we possess on the microbial flora of the soil and their activities. 5. Very little attempt is usually made to consider the soil micro-flora and its activities as a whole,—ordinarily, only isolated phenomena are considered. This can well be illustrated by the fact that nitrogen metabolism of bacteria is usually considered without a study of the carbon metabolism, as in the case of the so-called ammonification studies. 6. Last but not least, the fact that the soil is usually considered in the static condition of equilibrium, so far as soil microörganisms are considered, while it is actually in a dynamic condition, with the equilibrium continually shifted. This can be readily illustrated by referring to the well known Remy method, wherein a small quantity of soil is added to a definite amount of a sterile solution of known composition, then measuring the change that has taken place. This method is good enough for measuring the strength or activity of an unchangeable substance whether of chemical or biological origin (as in the case of enzymes), where the reaction is mono-molecular as far as the substance in question is concerned. In the case of a living soil flora, this method is entirely at fault, since the reaction is bi- tri-, and poly-molecular. It is assumed that the microörganisms do not multiply during the process of the reaction,—actually they not only multiply rapidly but some organisms utilize the products of others, with a host of antagonistic and stimulating substances formed in the process of the reaction, complicating greatly the study of the individual phenomena.

---

[1] Paper No. 56 of the Journal Series, New Jersey Agricultural Experiment Stations, New Brunswick, N. J. This paper will appear in RUTGER's COLLEGE STUDIES, vol. 1.

It was, therefore, deemed of greatest importance to take up first a study of the various functions used in the study of soil microbiology and interpret the results not only from a microbiological, but also from a chemical and physico-chemical viewpoint and particularly to investigate the variability of the methods themselves and of the soil as far as the function in question is concerned. The first 3 papers of this series deal with the question of numbers of soil micro-organisms, as determined by the plate method, and their relation to soil fertility.

THEORETICAL

In a bacteriological examination of the soil, the investigator is usually satisfied with a small number of samples taken from a limited area and regards the results obtained from a composite mixture of these samples, as accurate for the whole field. The variation between the samples, however, is of great enough importance to require very careful consideration. If this holds for an examination of the chemical soil constituents and crop production, it applies more so to a bacteriological soil analysis, where the very methods employed involve large errors which are usually not considered. The importance of calculating the mathematical error involved in an investigation has been recognized not only by the astronomer, physicist and chemist, but by the biologist as well. This allows us to judge not only the exactness of our investigation but also, when, on repeating the experment we obtain a different set of data, we could find out whether this change is only within the limits of error of the investigation or involves some new effect, which causes the change in the results.

There are only few reports on record dealing with the calculation of the mathematical error involved in the study of bacteriological activities in the soil, of which the two most important ones are those of Budinov (4) dealing with bacterial numbers in general and of Waynick (12) dealing with nitrification. Other attempts have been made to get some information on the variability in the actvities of microörganisms, but they either dealt with a function of doubtful importance in interpreting soil phenomena, e.g., ammonification, or no mathematical interpretation was attempted or both, as by Allison and Coleman (1).

Although this paper deals only with a study of numbers of microörganisms, the formulae presented are readily applied also to the variability in the study of other functions. The author felt justified in developing the formulae in this paper in view of the fact that it has not been done in any paper (except that of Budinov (4)) dealing with bacterial numbers and activities and even in the standard reference texts on statistics, such as that of Davenport (6). The need for the presentation of the derivation of formulae used in the mathematical interpretation of results became apparent, when, on examining the various texts, it was found that the statistician and mathematician used different formulae for calculating the average deviation. Davenport, for example, gives

$$= \sqrt{\frac{\Sigma\, d^2}{n}}$$

while Mellor (10) uses the formula

$$\sqrt{\frac{\Sigma\, d^2}{n-1}}$$

This may lead to a great deal of confusion, particularly in agricultural literature. Davenport does not state the method of the derivation of the particular formula and the justification for its use. In deriving the formula for the probable error of the mean, Davenport (6), Waynick (12), and others state that this is obtained by dividing the average deviation by the square root of n, which would make

$$Em = \pm\, 0.6745 \sqrt{\frac{\Sigma\, d^2}{n\,(n-1)}}$$

which would be the case only when the second formula for the average deviation is used.

The author has been assisted in developing the formulae for the average errors by the standard treatises on the theory of errors and methods of least squares by Bartlett (3), Carvallo (5), Woodward (14), and the more special texts, but particularly by a paper of Budinov (4), which appeared in 1910, in Russian, and which seemed to have escaped the attention of both European and American investigators.

To determine bacterial numbers in the soil, three methods are available: 1, the plate method; 2, the dilution method; and 3, the direct counting method. These methods are discussed in detail in the following paper and reasons are presented for selecting the plate method as the best one, under the present conditions. The limitations involved in the use of this method as well as details in technic will be fully discussed in that paper, so that we may limit ourselves here only to the method of the mathematical interpretation of the results. In brief, the soil samples are brought into the laboratory and are diluted with sterile tap water to give dilutions of 1:10, 1:1000 and 1:100,000; the first dilution is shaken for just 5 minutes by hand, the last dilution is used for the preparation of plates, in such a manner as to allow 40–200 colonies to develop on a plate. Where the soil is known to run much higher or much lower in numbers, the dilutions are made accordingly. Synthetic agar (egg-albumen) is used as a culture medium. The plates are incubated 7 days at 25°C., unless otherwise stated.

To count bacteria on the plate, three methods are commonly employed: (1) counting with the naked eye all the colonies on the plate, (2) use of counting plate, (3) use of microscope. The first is the most reliable and involves the least error. Of course where a large number of colonies are allowed to develop on the plate, which would necessitate the use of the counting plate or even the microscope there is greater probability of obtaining a greater variety of representative soil forms, and the errors involved in making high dilutions would be to some extent eliminated. The advantage is more than compensated by the fact that there is a great competition for the nutrients on the plate, and with lower dilutions, comparatively fewer bacteria will develop, as will be brought out in the following paper. To get a representative average, with the method of counting all bacteria on the plate, many plates have to be used. This as well as the fact that the counting of all the bacteria on the plate depends also a great deal on the sharpness of vision of the individual observers led various investigators to adopt one of the two other methods.

Since only the method of counting all colonies on the plate has been used in the experiments reported in this paper, we will limit ourselves primarily to a discussion of the errors involved in this method of counting. Some attention will also be given to the use of the counting plate, while the counting by means of the microscope will be omitted from this discussion. The mathematical considerations involved in the three methods of counting are quite similar, with only slight modifications. A mathematical interpretation of experimental results in the growth of plant is found in the papers of Wood (13), Wood and Stratton (14) and Davis (7). The tables given by Davenport (6) and Barlow (2) have been used in calculating the results.

Each set of observations involves occasional and constant errors, positive and negative from the mean. The occasional errors are eliminated as much as possible by the use of improved methods. The error of an observation is the difference between the result of the observation and the true value of the quantity. If the observation is s, the quantity measured q, the error x, then

$$x = s - q$$

The most probable value, which is the closest approximation to the true value that could be obtained from the series of observations is calculated by means of the method of least squares.

If the same quantity is measured many times and each time we obtain the difference (or residual) between the observed quantities and the mean value, then, by dividing the sum of the differences (residuals) by the number of observations, we obtain the mean arithmetical error. The residual bears the same relation to the true error that the most probable value bears to the true value. With the increase in the number of observations, the most probable value approaches the true value, while residuals approach the corresponding true errors. In this case, the limit of the error is 0, since the positive and negative declinations from the mean are occasional errors, not counting, of course, the constant errors.

If n plates are used for making a quantitative bacteriological count of a given soil, a is the number of colonies developing on the plates, z is the true value, while z_n is the most probable value obtained from counting n plates, or the arithmetical mean value, then we have

$$z_n = \frac{\Sigma\, a}{n}$$

In other words, the mean (z_n) is found by dividing the sum of all the determinations by the number of determinations. Since the true value is z, each determination varies from the true value by

$$x_1 = z - a_1;\ x_2 = z - a_2;\ x_3 = z - a_3 \cdots\cdots\cdots\ x_n = z - a_n$$

If
$$\frac{\Sigma\, x}{n} = \Delta\, z$$

then
$$z = \frac{\Sigma\, a}{n} + \frac{\Sigma\, x}{n} = z_n + \Delta\, z$$

$\Delta\, z$ is the error of the mean from the true value of the given quantity. In view of the fact that the error can be either positive or negative, the error of the mean squared can then be expressed as the square of the sum of the errors divided by their number.

$$(\Delta\, z)^2 = \left(\frac{\pm x_1 \pm x_2 \pm x_3 \cdots\cdots\cdots \pm xn}{n}\right)^2$$

By expanding and combining this equation, we find

$$(\Delta z)^2 = \frac{\Sigma\, x^2}{n^2}$$

as n increases indefinitely. The average error of a single observation from the arithmetical mean or standard deviation (σ) is usually assumed to be equal to $\sqrt{\frac{\Sigma x^2}{n}}$, in that case $\Delta z = \frac{\sigma}{\sqrt{n}}$. The standard deviation is a measure of the reliability of any one result; the chances are even that any one determination taken at random will differ from the average by this deviation. In other words, the error of the mean (Δz) is inversely proportional to the square root of the number of observations; the error of the mean of 100 observations will be 10 times less than the average error of single observations. The laws of chance cannot be expected to give normal results with small numbers, therefore, the larger the number of observations, the more reliable is the average obtained. This holds true, in the following investigations, both for the number of plates used in counting microörganisms and for the number of soil samples taken from one particular plot.

Since we know only the arithmetical mean (z_n) and not the true value (z), we have

$$z = z_n + \Delta z, \text{ then}$$
$$x_1 = z_n + \Delta z - a_1 = (z_n - a_1) + \Delta z$$
$$x_2 = z_n + \Delta z - a_2 = (z_n - a_2) + \Delta z$$
$$\cdots\cdots\cdots\cdots\cdots\cdots$$
$$\cdots\cdots\cdots\cdots\cdots\cdots$$
$$x_n = z_n + \Delta z - a_n = (z_n - a_n) + \Delta z$$

By squaring and adding we find

$$\Sigma x^2 = \Sigma (z_n - a)^2 + n \Delta z^2$$

Dividing the above equation by n,

$$\frac{\Sigma x^2}{n} \quad \frac{\Sigma (z_n - a)^2}{n} \cdot + \Delta z^2$$

Substituting σ^2 for the first part of the equation and $\frac{\sigma}{\sqrt{n}}$ for Δz, we find that

$$\sigma^2 = \frac{\Sigma (z_n - a)^2}{n} + \frac{\sigma 2}{n}, \text{ or}$$

$$\sigma = \sqrt{\frac{\Sigma (z_n - a)^2}{n - 1}}, \text{ therefore}$$

$$\Delta z = \sqrt{\frac{\Sigma (z_n - a)^2}{n (n - 1)}}$$

The average deviation is thus found to be different from that originally assumed. The probable error of the individual observation or arithmetical mean is obtained by multiplying the above formulae by ± 0.6745:

$$Em = \pm 0.6745 \sqrt{\frac{\Sigma (z_n - a)^2}{n (n - 1)}}$$

Since for a single observation, $\sqrt{n} = 1$, the probable error is

$$\Sigma s = \pm 0.6745 \times \sigma$$

Approximately two-thirds of the determinations are expected to lie on both sides of the mean, within that deviation.

The probable error of the standard deviation is obtained from the formula $\pm 0.6745 \frac{\sigma}{\sqrt{2n}}$. The coefficient of variability (C.V.) according to Davenport (6) is the percentage ratio of the standard deviation (σ) from the mean. The probable error of the coefficient of variability $= \pm 0.6745 \frac{\text{C.V.}}{\sqrt{2n}}$. By the use of formulae for the calculations of the errors of observation, we can now figure out the error of the mean arithmetical value of the plate counts. The formula used for the standard deviation in the following investigations, is the second derived formula, namely

$$(1) \qquad \sigma = \sqrt{\frac{\Sigma\,(z_n - a)^2}{n - 1}}$$

However, for comparison with the work based on Davenport's formula, a formula is given whereby the results obtained from the derived formula (1) can easily be transformed into results that would be obtained by using the Davenport formula (2); m (in other words σ, according to Davenport) $= \sigma \sqrt{\frac{n - 1}{n}}$,

$$(2) \qquad m\,(\sigma) = \sqrt{\frac{\Sigma\,(z_n - a)}{n}}$$

If N is the total number of colonies, a the number on each plate, and u the amount of dilution, then

$$N = au$$

In the mathematical interpretation of the results obtained by any soil bacteriological function, we must consider two sets of variables: that of the soil and that of the method. Both of these are of equal importance: the first has been well illustrated in the nitrification studies of Waynick (12) and the second in the mathematical analysis of the quantitative determination of bacteria in milk by Budinov (4). Waynick (12) dealt with the question of nitrification, and the method commonly employed gives rather constant results, although something will have to be said about the actual true value of the method as interpreting a biological function. In the study of such a function as the determination of bacterial numbers in the soil, the method itself, as pointed out above, is quite a variable factor,—it should therefore be considered first, before the variability of the soil itself, as far as numbers of microörganisms are concerned is considered.

VARIABILITY OF THE PLATE METHOD FOR COUNTING SOIL MICROÖRGANISMS

For the study of the variability of the method, a sample of soil was taken from a quantity of soil kept under optimum moisture conditions for the last eight months. A dilution of 100,000 was made and 62 plates were poured. A few plates were also prepared from a dilution of 1,000 of the same soil. The plates made from the first dilution were used for the variability of the method by counting all colonies, while the plates obtained with the low dilution were counted utilizing the counting plate.

TABLE 1

The use of the counting plate in determining the numbers of microörganisms in the soil, by the plate method

COLONIES	$(z_n - a)$	$(z_n - a)^2$	COLONIES	$(z_n - a)$	$(z_n - a)^2$
51	+17.4	302.76	31	− 2.6	6.76
56	+22.4	501.76	17	−16.6	275.56
48	+14.4	207.36	20	−13.6	184.96
59	+25.4	645.16	19	−14.6	213.16
52	+18.4	338.56	19	−14.6	213.16
41	+ 7.4	54.76	22	−11.6	134.56
39	+ 5.4	29.16	24	− 9.6	92.16
34	+ 0.4	0.16	36	+ 2.4	5.76
36	+ 2.4	5.76	44	+10.4	108.16
29	− 4.6	21.16	57	+23.4	547.56
33	− 0.6	0.36	41	+ 7.4	54.76
24	− 9.6	92.16	49	+15.4	237.16
25	− 8.6	73.96	42	+ 8.4	70.56
14	−19.6	384.16	38	+ 4.4	19.36
23	−10.6	112.36	26	− 7.6	57.76
18	−15.6	243.36	29	− 4.6	21.16
19	−14.6	213.16	28	− 5.6	31.36
23	−10.6	112.36	24	− 9.6	92.16
35	+ 1.4	1.96	32	− 1.6	2.56
43	+ 9.4	88.36	34	+ 0.4	0.16
19	−14.6	213.16	23	−10.6	112.36
28	− 5.6	31.36	22	−11.6	134.56
52	+18.4	338.56	16	−17.6	309.76
55	+21.4	457.96	31	− 2.6	6.76
36	+ 2.4	5.76	24	− 9.6	92.16
49	+15.4	237.16	27	− 6.6	43.56
47	+13.4	179.56			
51	+17.4	302.76	2016*	− 5.0	8357.40*
33	− 0.6	0.36			
36	− 2.6	6.76			
34	+ 0.4	0.16			
34	+ 0.4	0.16			
26	− 7.6	57.76			
39	+ 5.4	29.16			

Mean = 33.6 ± 1.03

σ = ±11.9 ±0.74

$C.V.$ = 35.4 ± 2.18 per cent

Δz = ±1.53

Em = 3.06 per cent

* Totals of both columns.

In this study all the colonies, besides the fungi, were counted without any attempt made to differentiate between the bacteria and actinomycetes. Table 1 contains the results obtained from the use of the counting plate. The microörganisms were so numerous, that they were counted at the end of 3 days.

When the plates are counted by means of the counting plate, we must keep in mind that we have to trust the firm preparing the plate, in its accuracy. The method of calculating the error applies to this method in a way similar to the one worked out for counting the whole plate, only here we count various sections of the plate.

The first column represents the number of colonies, the second, $(z_n\text{-}a)$, gives the errors obtained by subtracting the individual counts from the arithmetical mean, the third column, $(z_n\text{-}a)^2$, contains the squares of the errors.

The average of the 60 counts on the plate is 33.6. The sum of the squares is found to be 8,357.40, therefore the average deviation is, for each count:

$$\sigma = \sqrt{\frac{\Sigma\,(z_n - a)^2}{n-1}} = \sqrt{\frac{8{,}357.40}{59}} = \pm 11.9$$

$$C.V. = \frac{11.9 \times 100}{33.6} = 35.4 \text{ per cent}$$

The error of the mean observed value is

$$\Delta z = \frac{\sigma}{\sqrt{n}} = \pm 1.53$$

The most probable error is then equal to

$$Em = \pm 1.53 \times 0.6745 = \pm 1.03 = 3.06 \text{ per cent}$$

Since the number of the sections on the plate was 60 and the dilution 1000, the total number of microörganisms, exclusive of fungi, obtained by this method is $1000 \cdot 60 \cdot (33.6 \pm 1.03) = 2{,}016{,}000 \pm 61{,}800$ per gram of moist soil.

It is interesting to note that the sample gave in the same period of incubation, an average of 561 colonies with 10,000 dilution and 64 colonies with 100,000 dilution.

The second experiment deals with the variability involved in the use of a large number of plates, with a dilution commonly employed in determining the numbers of microörganisms in the soil, whereby only between 40 to 200 colonies are allowed per plate, and a long enough period of incubation is employed to allow a maximum development of microörganisms. Tables 2 and 3 contain the counts made on the same soil used in the previous experiment, with a dilution of 100,000. The plates were incubated for 7 days at 25–27°C.

In counting the colonies developing on the plates, first all colonies, except fungi, were counted, then the actinomycetes were determined separately.

The arithmetical mean of all the 62 plates, considering at first the bacteria (in other words, bacteria+actinomycetes) is 73.68.

$$= \sqrt{\frac{8458.5488}{61}} = \pm 11.78$$

$$C.V. = \frac{11.78 \times 100}{73.68} = 15.99 \text{ per cent}$$

$$\Delta z = \frac{\sigma}{\sqrt{n}} = \frac{11.78}{\sqrt{62}} = \pm 1.50$$

$$Em = \pm 1.50 \times 0.6745 = \pm 1.012 = 1.36 \text{ per cent}$$

The total number of microörganisms, exclusive of fungi, is then found to be (73.68 ± 1.012) × 100,000 = 7,368,000 ± 101,200, without considering the moisture of the particular soil.

When the coefficient of variation and the most probable error of the two methods of counting bacteria are compared, we find that it is less than a half, by the use of a large number of plates and counting all colonies, than in the case of counting the colonies on the Petri dish by means of a counting plate. For this reason and other reasons given above, the method of making high dilutions, using a large number of plates and counting all the colonies on the plate is to be preferred.

When the actinomyces colonies are considered, we find the arithmetical mean to be 24.85.

$$= \sqrt{\frac{1149.6950}{61}} = \pm 4.34$$

$$C.V. = \frac{4.34 \times 100}{24.85} = 17.47 \text{ per cent}$$

$$\Delta z = \frac{4.34}{\sqrt{62}} = \pm 0.55$$

$$Em = \Delta 0.55 \times 0.6745 = \pm 0.371 = 1.50 \text{ per cent}$$

The *C. V.* and most probable error are found to be for actinomycetes somewhat higher than that for the number of bacteria plus actinomycetes. A possible explanation for that will be suggested below. Attention may be called here to the work of Johnstone (9) on the probable error involved in a bacteriological analysis of the fecal pollution of shell fish. The frequency curve obtained was highly asymmetrical. A dilution of 250 cc. was prepared and 20 plates were counted. The highest frequency ran with the lowest counts. Since the calculation of the probable value of a measure presupposes a symmetrical distribution (Gaussian), it is not legitimate to apply the probable error to a highly asymmetrical distribution. Michael (10) suggests that in that case some influence other than "chance" is operative, and a function should be substituted whose distribution is Gaussion, which will enable the separation. The function suggested is the logarithmic value. The most probable number of the bacteria will then be not the arithmetical mean, but the geometric mean. This may be true enough, but another error is overlooked, namely the use of too high a dilution, which allows too few bacteria to develop on the plate. It will be pointed out in the following paper (as shown also by other investigators) that the number of bacteria allowed per plate should be between 40 and 200. Where larger numbers of colonies develop, there is apt to be overcrowding; with fewer numbers, the samples are apt to be unrepre-

TABLE 2

The variability of numbers of bacteria in the soil as determined by the common plate method

COLONIES	$(z_n - a)$	$(z_n - a)^2$	COLONIES	$(z_n - a)$	$(z_n - a)^2$
69	− 4.68	21.9024	60	−13.68	187.1424
74	+0.32	0.1024	62	−11.68	136.4224
71	− 2.68	7.1824	99	+25.32	641.1024
69	− 4.68	21.9024	94	+20.32	412.9024
64	− 9.68	93.7024	81	+ 7.32	53.5824
71	− 2.68	7.1824	86	+12.32	151.7824
67	− 6.68	44.6224	75	+ 1.32	1.7424
56	−17.68	312.5824	85	+11.32	128.1424
59	−14.68	215.5024	67	− 6.68	44.6224
68	− 5.68	32.2624	74	+ 0.32	0.1024
73	− 0.68	0.4624	97	+23.32	543.8224
87	+13.32	177.4224	88	+14.32	205.0624
70	− 3.68	13.5424	83	+ 9.32	86.8624
75	+ 1.32	1.7424	60	−13.68	187.1424
77	+ 3.32	11.0224	71	− 2.68	7.1824
68	− 5.68	32.2624	72	− 1.68	2.8224
76	+ 2.32	5.3824	66	− 7.68	58.9824
84	+10.32	106.5024	74	+ 0.32	0.1024
76	+ 2.32	5.3824	68	− 5.68	32.2624
106	+32.32	1044.5824	81	+ 7.32	53.5824
74	+ 0.32	0.1024	67	− 6.68	44.6224
81	+ 7.32	53.5824	89	+15.32	234.7024
76	+ 2.32	5.3824	69	− 4.68	21.9024
98	+24.32	591.4624	87	+13.32	177.4224
70	− 3.68	13.5424	78	+ 4.32	18.6624
52	−21.68	469.0224	62	−11.68	136.4224
63	−10.68	114.0624	55	−18.68	348.9424
59	−14.68	215.5024			
72	− 1.68	2.8224	4568*	+ 0.84	8458.5488*
73	− 0.68	0.4624			
51	−22.68	514.3824			
74	+ 0.32	0.1024			
56	−17.68	312.5824			
83	+ 9.32	86.8624			
76	+ 2.32	5.3824			

Mean = 73.68 ±1.01
σ = 11.78 ±0.72
C. V. =15.99 ± 0.97 per cent
Δz = ± 1.50
Em = 1.36 per cent

* Totals of both columns.

sentative. Out of the 20 plates used by Johnstone (9), 19 contained less than 30 colonies per plate; this accounts for the fact that an asymmetrical curve has been obtained and accounts for the influence other than "chance" mentioned by Dr. Michael (11).

TABLE 3

The variability of numbers of actinomycetes in the soil as determined by the common plate method

COLONIES	$(z_n - a)$	$(z_n - a)^2$	COLONIES	$(z_n - a)$	$(z_n - a)^2$
21	−3.85	14.8225	18	− 6.85	46.9225
23	−1.85	3.4225	24	− 0.85	0.7225
29	+4.15	17.2225	27	+ 2.15	4.6225
28	+3.15	9.9225	27	+ 2.15	4.6225
20	−4.85	23.5225	23	− 1.85	3.4225
23	−1.85	3.4225	32	+ 7.15	51.1225
18	−6.85	46.9225	28	+ 3.15	9.9225
25	+0.15	0.0225	33	+ 8.15	66.4225
26	+1.15	1.3225	24	− 0.85	0.7225
19	−5.85	34.2225	18	− 6.85	46.9225
25	+0.15	0.0225	28	+ 3.15	9.9225
24	−0.85	0.7225	27	+ 2.15	4.6225
23	−1.85	3.4225	22	− 2.85	8.1225
24	−0.85	0.7225	32	+ 7.15	51.1225
28	+3.15	9.9225	23	− 1.85	3.4225
26	+1.15	1.3225	26	+ 1.15	1.3225
30	+5.15	26.5225	26	+ 1.15	1.3225
21	−3.85	14.8225	25	+ 0.15	0.0225
20	−4.85	23.5225	31	+ 6.15	37.8225
28	+3.15	9.9225	24	− 0.85	0.7225
23	−1.85	3.4225	34	+ 9.15	83.7225
22	−2.85	8.1225	29	+ 4.15	17.2225
25	+0.15	0.0225	38	+13.15	172.9225
28	+3.15	9.9225	31	+ 6.15	37.8225
23	−1.85	3.4225	26	+ 1.15	1.3225
18	−6.85	46.9225	28	+ 3.15	9.9225
20	−4.85	23.5225	25	+ 0.15	0.0225
22	−2.85	8.1225			
23	−1.85	3.4225	1541*	+ 0.30	1149.6950*
19	−5.85	34.2225			
24	−0.85	0.7225			
18	−6.85	46.9225			
23	−1.85	3.4225			
24	−0.85	0.7225			
19	−5.85	34.2225			

Mean = 24.85 ± 0.37
σ = 4.34 ± 0.27
$C. V.$ = 17.47 ± 1.14
Δz = ±0.55
Em = 1.50 per cent

* Totals of both columns.

VARIABILITY OF THE SOIL IN RELATION TO NUMBERS OF MICROÖRGANISMS

To study the variability of the soil itself as far as bacterial numbers are concerned, a field plot was selected. The plot is one of the experimental plots at the College Farm at New Brunswick, used by the Soil Department of this station for the study of utilization of nitrogenous fertilizers. The particular plot received annually for the last 14 years, 320 pounds $NaNO_3$ per acre, in addition to minerals. An outline of the plots and the rotation of crops used will be found in the following paper. In 1921, the plot was in timothy. Samples were taken 2 to 3 weeks after the crop had been harvested.

TABLE 4

Bacterial colonies in the upper 6 inches of soil of Plot 9A, dilution: 1–100,000, and moisture content of samples

NUMBER OF SAMPLE	COLONIES	MOISTURE CONTENT	NUMBER OF SAMPLE	COLONIES	MOISTURE CONTENT
		per cent			*per cent*
1	97.0 ± 2.65	12.3	30	186.8 ± 6.27	12.5
2	99.2 ± 3.34	12.1	31	128.3 ± 5.74	14.5
3	86.3 ± 4.50	12.3	32	148.8 ± 3.71	13.6
4	161.0 ± 6.88	13.2	33	116.7 ± 5.53	13.7
5	132.2 ± 7.35	14.3	34	112.8 ± 5.50	13.4
6	119.4 ± 5.84	14.3	35	138.5 ± 4.40	11.8
7	102.5 ± 4.39	13.7	36	146.6 ± 4.01	12.9
8	121.2 ± 5.96	13.5	37	181.8 ± 1.58	12.6
9	111.8 ± 7.52	12.9	38	151.5 ± 3.25	12.6
10	133.7 ± 3.13	13.2	39	175.4 ± 5.26	13.0
11	146.2 ± 2.17	13.2	40	132.7 ± 3.79	12.4
12	118.0 ± 5.34	13.6	41	99.3 ± 2.01	12.8
13	135.0 ± 6.42	13.5	42	86.5 ± 3.64	10.9
14	136.8 ± 5.15	12.2	43	95.7 ± 3.08	11.1
15	164.7 ± 3.75	13.6	44	89.7 ± 4.71	11.1
16	120.0 ± 3.86	12.5	45	107.7 ± 2.27	10.7
17	132.2 ± 3.44	13.2	46	113.7 ± 6.79	12.2
18	177.8 ± 7.34	14.8	47	109.3 ± 3.93	11.8
19	189.8 ± 6.76	12.9	48	96.8 ± 4.68	13.0
20	111.5 ± 4.81	13.2	49	89.5 ± 2.96	13.2
21	125.5 ± 6.49	12.1	50	164.6 ± 2.63	11.3
22	101.3 ± 3.29	11.6	51	112.5 ± 7.44	12.8
23	226.8 ± 11.82	13.3			
24	167.6 ± 8.11	13.1		Mean = 128.9 ± 2.97	12.8 ± 0.09
25	111.2 ± 5.14	11.6		σ = 31.5 ± 2.1	0.9 ± 0.10
26	100.3 ± 7.74	11.4		*C. V.* = 24.44 ± 1.63 per cent	7.66
27	92.8 ± 3.12	12.8		Δz = ±4.4	±0.14
28	118.2 ± 3.60	14.4		*Em* = 2.30 per cent	0.7
29	147.7 ± 2.06	14.3			

Several rather heavy rainfalls preceded the sampling of the plot by about a week.

The plot is $\frac{1}{20}$ of an acre in size, 66 x 33.3 feet. The samples were taken by means of a 1-inch sampling tube to a depth of 6½ inches, 3–4 feet from the edge, and about 5 x 8 feet apart, 6 rows in width and 8 rows in length. The soils were plated out soon after they were brought into the laboratory, six plates were used for each sample. A larger number of plates should have been used, but, in that case, not all the samples could have been plated out in one day; it was thought that the error involved in plating out the different samples on the different days would be greater than the use of a smaller number of plates. Due to lack of space, only the arithmetical mean for each sample is reported in the columns. The results and the moisture content of the samples are given in table 4. The extremes in numbers of microörganisms (bacteria and actinomycetes) are found to be 86.3 ± 4.50 and 226.8 ± 11.82 colonies, which represent 8,630,000 ± 450,000 and 22,680,000 ± 1,182,000 per gram of moist soil. Were the results for bacterial numbers in all the other nitrogen plots reported here (see following paper), they would be found to come, with one or two exceptions, within this range. In other words, *wherever determinations of bacterial numbers in the soil are based only on one sample, the results are worthless, due to the great variability of the soil in respect to numbers of microörganisms.*

There was in some cases an evident correlation between the position of the sample and the number of microörganisms. The samples taken at the edges of the plot, particularly in the corners, gave lower numbers than the samples coming from the middle of the plot. This may be due, in some cases at least, to an uneven distribution of the fertilizer. Perhaps it may be due, in some cases, to the lower moisture content. This would lead us to conclude that the determination of numbers of microörganisms in the soil should be based not on one but on a number of samples taken from various parts of the particular field.

The combined variability of the method and the soil

With these considerations in mind, we can now proceed to the development of a practical method for determining the number of microörganisms in the soil which would allow both for the variability of the plate method and that of the soil, without involving too great an excess of time and energy and which could be used in routine work. For the following experiment, a check plot was used, which had received no fertilizer application at all for the last 14 years.

Samples were taken from five different parts of the plot, each sample representing a mixture of three borings taken 1–2 feet apart. The samples were thoroughly mixed, placed in sterile jars, and plated out as soon as brought into the laboratory. Ten plates were used for each sample, so that fifty plates were poured for the five samples taken from the same plot. A dilution of 100,000 was used in all cases. The plates were incubated for 7 days at 25°C., then the microörganisms were counted. The results are tabulated in table 5, the clear spaces differentiating the different samples.

The average of all 50 plates obtained from the five samples of the same soil is $\frac{4178}{50} = 83.46 \pm 1.5$ for the total microörganisms, exclusive of the fungi; $\frac{556}{50} = 11.3 \pm 0.3$ for the actinomycetes; and $\frac{138}{50} = 2.76 \pm 0.16$ for the fungi.

The probable error for the number of microörganisms, exclusive of fungi, is 1.8 per cent. Since the dilution is 100,000 and the moisture content of

TABLE 5

Numbers of microörganisms in the soil, as shown by the plate method

BACTERIA AND ACTINOMYCETES		ACTINOMYCETES		FUNGI	
Colonies		*Colonies*		*Colonies*	
94	79	9	7	6	3
84	83	8	13	3	6
73	103	11	14	2	3
61	98	10	12	2	5
99	76	9	10	2	2
83	76	15	12	3	3
67	112	9	13	3	4
77	101	15	15	3	2
65	84	7	12	6	3
91	56	11	11	2	2
64	106	13	14	2	4
96	101	10	16	0	3
81	89	11	13	2	3
53	84	8	12	4	5
96	116	7	14	4	1
69	76	8	12	4	1
72	82	15	11	7	1
62	93	13	9	1	1
69	76	12	15	7	0
77	84	7	9	3	1
106	52	11	4	2	2
93	78	9	17	4	1
98	61	9	11	2	1
87	93	12	13	0	1
91	106	6	21	4	2
Total = 4173		Total = 565		Total = 138	
Mean = 83.46 ± 1.5		Mean = 11.3 ± 0.30		Mean = 2.76 ± 0.16	
σ = 15.69 ± 1.07		σ = 3.16 ± 0.20		σ = 1.73 ± 0.11	
C. V. = 18.8 ± 1.27 per cent		*C. V.* = 27.96 ± 1.89 per cent		*C. V.* = 62.67 ± 4.23 per cent	
$\Delta z = \pm 2.22$		$\Delta z = \pm 0.45$		$\Delta z = \pm 0.245$	
Em = 1.8 per cent		*Em* = 2.7 per cent		*Em* = 5.8 per cent	

the particular soil is 10 per cent, the total number of microörganisms, exclusive of fungi, in the particular plot, on the basis of dry soil, is found by multiplying the colonies by 100,000 and by $\frac{10}{9}$:

$$(83.46 \pm 1.5) \times 100{,}000 \times \frac{10}{9} = 9{,}273{,}333 \pm 166{,}667$$ (true within 1.8 per cent).

In the case of actinomycetes, the probable error of the mean is 2.7 per cent. By multiplying by 100,000 and $\frac{10}{9}$, we obtain the most probable number of actinomycetes, within the limits of the methods used for their determination, as 1,255,556 ± 33,333 per gram of dry soil.

The error of the determination of actinomycetes is thus found to be once and a half as great as that for the determination of total number of microorganisms exclusive of the fungi. This can be readily explained by the fact that there are greater errors involved in counting actinomycetes colonies than in counting the total number of colonies: in the last case all colonies are counted, the fungi being distinguished very readily, and, given a long enough period of incubation and little chance for overcrowding, particularly by spreaders and fungi, the colonies can be quite accurately counted; in the case of actinomycetes, we add the error of mistaking sometimes an actinomyces for a bacterial colony and *vice versa*. Of course, the fact that the number of actinomycetes is less than the total number of colonies will tend to increase the probable error.

The most probable number of fungus colonies is found to be

$$2.76 \pm 0.16$$

The most probable value of the total number of fungi in the particular soil is then:

$(2.76 \pm 0.16) \times 100{,}000 \times \frac{10}{9} = 306{,}667 \pm 17.778$ per gram of dry soil true within 5.8 per cent.

There is a tremendous jump in the probable error of determining fungi by this particular method. While the most probable error is true for the total number of microörganisms, exclusive of fungi, within 1.8 per cent and for the actinomycetes within 2.7 per cent, it is true for the fungi only within 5.8 per cent, a range more than three times as great as for the other soil microörganisms. This cannot be explained, as was done in the case of the actinomycetes, by the errors made in recognizing the colonies, since the fungus colonies are well recognized, particularly when the plates are incubated 7 days.

The reason for this large error lies elsewhere, namely in the inefficiency of the plate method for the determination of numbers and activities of fungi in the soil. Since the fungi exist in the soil both in the form of spores and in the form of aerial mycelium, and because of the great abundance of spores formed in a single head of an *Aspergillus*, *Penicillium* and *Mucor*, the error involved is greatly increased: while one small clump of soil may be free from sporulating mycelium, another clump very near to that may contain even one sporulating piece of mycelium which will account for the error. As a matter of fact all we need to do for a lucid explanation of this phenomenon is to point out the upper and lower groups of colonies of fungi in the column in the table.

But another important factor is to be considered, that of the small number of fungi developing on the plates primarily designed, both as to medium and dilution, for the counting of bacteria and actinomycetes.

This factor is sufficient to omit entirely the counting of fungi on the common plate used for the counting of bacteria and actinomycetes. However, it has been used in the experiments submitted in the following paper, due to the fact that some information was obtained even with such an error, which is sufficient to condemn it, for the particular study made. But this is only a specific instance, while as a general method of procedure it is not to be recommended. If it is designed to obtain more or less accurate information on the fungus numbers in the soil, the method proposed below should be used.

To turn back to the table, we find that interesting observations can be made on comparing the number of colonies obtained from each soil sample. For this we must separate the five groups, of ten plates each, which represent the five composite samples taken from the same field. We will limit ourselves in this instance, only to the total number of colonies, exclusive of the fungi, since this is the least variable factor and, therefore, the most reliable. On averaging the five groups, we obtain the following number of colonies for each sample: 79.4, 73.9, 91.4, 92.5, 80.1. The total average is 83.46.

The most probable errors for these 5 groups are:

± 2.77, ± 2.99, ± 2.16, ± 3.92, ± 3.31, respectively equivalent to

3.50 per cent, 4.05 per cent, 2.36 per cent, 4.25 per cent, 4.11 per cent

The error is found to be much greater than when it is based upon a count of 50 plates representing all the five samples. When we calculate the probable error of these five samples, we find it to be equivalent to ±2.45 or 2.93 per cent. By limiting ourselves to fewer and fewer samples we get a larger and larger error; by taking fewer and fewer plates for each sample we also increase our error. By increasing the number of samples taken and the number of plates used for each sample, we appreciably decrease the probable error.

The reason for the indefiniteness of the results obtained from counting the number of microörganisms, and lack of correlation between these results and actual crop production is to be looked for in the fact that the great majority of determinations were based upon one or two samples, in many instances not even composite samples, and 2–4 plates were used for making the determinations. Among other errors involved we must point to the lack of proper culture media (not standard in composition), in some cases a short incubation period, whereby not all the colonies were allowed to develop, and errors in the technic. These questions will be taken up in detail in the following paper.

As an example of the error usually obtained in a quantitative bacteriological soil analysis, we may point to the excellent work of Hiltner and Störmer who stated to have obtained an error, in the determination of numbers of bacteria in the soil, of 8–10 per cent and in some cases 15 per cent. Only one sample was taken (representing however a thorough mixture of several samples) and 4 plates were used. It is interesting to note that these investigators, as long as 20 years ago, recognized the importance of the large error involved, but they tried to correct it by improving the technic. The use of a larger number of plates for the reduction of the error has been recognized, but not carried out.

The question whether a quantitative determination of the number of microorganisms can be taken as a function of soil bacteriology will be answered only in the following paper. However, on the basis of the mathematical interpretation of the results, we might state here that the determination of the total number of microörganisms based on a number of soil samples with a number of plates used for each sample and proper technic, whereby the probable error is reduced to less than 2 per cent, seems to offer an appropriate biological function.

THE DETERMINATION OF NUMBERS OF FUNGI IN THE SOIL

The mathematical study of the distribution of fungi in the soil reported in the last table coupled with other considerations considered by the writer elsewhere, would lead us to condemn the plate method for counting fungi as a method for the study of the activities or even distribution of these organisms in the soil. However, another method could be suggested here, namely the

TABLE 6

The numbers of fungi in the soil

COLONIES	$(z_n - a)$	$(z_n - a)^2$
36	+ 4.8	23 04
27	− 4.2	17 64
38	+ 7.8	60.84
30	− 1.2	1.44
24	− 7.2	51.84
26	− 5.2	27.04
27	− 4.2	17.64
21	−10.2	104.04
26	− 5.2	27.04
24	− 7.2	51.84
35	+ 3.8	14.44
37	+ 5.8	33.64
20	−11.2	125.44
28	− 3.2	10.24
37	+ 5.8	33.64
41	+ 9.8	96.04
34	+ 2.8	7.84
32	+ 0.8	0.64
39	+ 7.8	60.84
31	− 0.2	0.04
23	− 8.2	67.24
25	− 6.2	38.44
26	− 5.2	27.04
33	+ 1.8	3 24
29	− 2.2	4.84
37	+ 5.8	33.64
29	− 2.2	4.84
26	− 5.2	27.04
44	+12.8	163.84
32	+ 0.8	0.64
36	+ 4.8	23.04
42	+10.8	116.64
35	+ 3.8	14.44
28	− 3.2	10.24
36	+ 4.8	23.04
32	+ 0.8	0.64
28	− 3.2	10.24
32	+ 0.8	0.64
33	+ 1.8	3.24
29	− 2.2	4.84
1248*	+ 1.0	1343.00*

Mean = 31.2 ± 0.63

$\sigma = \pm 5.87 \pm 0.45$

C. V. = 18.8 ± 1.42 per cent

$\Delta z = 0.93$

Em = 2.0 per cent

* Totals of both columns.

use of media which will allow only a development of fungi, but not of other microörganisms, so that the final dilution could be made low enough, whereby 25 to 100 colonies of fungi could develop on each plate. The period of incubation should be between 2 and 3 days, at 25°C. Longer periods will favor overgrowth, while a shorter period will not allow all fungi to develop. A medium like raisin agar, which is very acid in reaction or a synthetic medium having a reaction of pH = 3.5–4.0 will be found appropriate, while the dilution may be 0.01 of that used for the plating out of bacteria. Under these conditions, the probable error for the determination of numbers of fungi (spores and pieces of mycelium) is appreciably reduced.

The following table will give som eresults obtained by this method. The same soil used in the last experiment has been used for this study. A dilution of 1000, an incubation period of 3 days at 25–27°C. and raisin agar were employed. The results are self explanatory and need not be discussed any further. The marked reduction in the probable error shows that fungi can be determined quantitatively in the soil only when a special acid medium has been used so that the bacteria and actinomycetes are eliminated, and only the fungi develop on the plate. A low enough dilution should be used to allow 20 to 100 colonies per plate.

SIGNIFICANCE OF A QUANTITATIVE BACTERIOLOGICAL ANALYSIS OF SOIL

Since it is often impossible to report all the details connected with the determinations of numbers of microörganisms, as far as all individual plates for every sample are concerned, it is suggested to designate, by a definite figure, the "weight" or importance to be attached to the results, in addition, of course, to the probable error. The weight to be attached to each sample should be the logarithm of the number of plates used in determining the numbers of microörganisms in a particular sample. The weight to be attached to the numbers of soil samples taken from a particular field or plot should be in direct proportion to that number. Each soil sample should be a composite of two or three samples, if possible. We will then have the following series:

[illegible]	"WEIGHT"			
	1 sample	2 samples	3 samples	10 samples
1	0	0	0	0
2	0.30	0.60	0.90	3.0
3	0.48	0.96	1.44	4.8
4	0.60	1.20	1.80	6.0
5	0.70	1.40	2.10	7.0
6	0.78	1.56	2.24	7.8
7	0.85	1.70	2.55	8.5
8	0.90	1.80	2.70	9.0
9	0.95	1.90	2.85	9.5
10	1.00	2.00	3.00	10.0

A weight less than 1.0 is practically worthless, a weight of 1.0 to 2.0 is fair, 2–3 is good, 3–5 is very good. A higher weight than 5.0, particularly if the samples are composite may involve an excessive amount of work, but will, of course give still more reliable results.

SUMMARY

1. The variability of the common method employed for quantitative determination of microörganisms in the soil is too high, when only 2–3 plates are used, and too much reliability cannot be attached to the results.
2. Only by the use of a large number of plates and by making an accurate determination of the probable error involved can we state definitely how much weight we may attach to the results obtained from a quantitative examination of the microörganisms in the soil.
3. The dilution commonly employed for counting bacteria and actinomycetes is too high for the counting of fungi, so that the results obtained from counting fungi on the same plate with the bacteria and actinomycetes are worthless for most soils. Special acid media, whereby only fungi will develop combined with a low dilution (about 100 of that used for counting bacteria) will give more reliable results.
4. The variability of the soil samples taken from the field is too high, we should be able to attach great importance to determinations based upon a single sample.
5. A number of samples should be taken from each field; these are then composited into fewer samples, which should be used for the bacteriological studies. Even then, we should determine the probable error of our function from a large number of individual samples.
6. By combining a relatively large number of plates with a large number of soil samples, we can work out the probable error involved in a quantitative determination of the microörganisms in the soil.
7. A set of figures for the designation of the "weight" to be attached to the determinations based on the number of plates and soil samples has been suggested.

The writer wishes to express his indebtedness to Mr. J. S. Joffe and Mr. R. L. Starkey for aid in taking the samples and in pouring the plates.

REFERENCES

(1) ALLISON, F. E., AND COLEMAN, D. A. 1917 Biological variations in soil plots as shown by different methods of sampling. *In* Soil Sci., v. 3, p. 499–514.

(2) BARLOW, PETER 1914 Tables of Squares, Cubes, Square roots, Cube roots, Reciprocals. Spon, London.

(3) BARTLETT, D. P. 1915 General Principles of the Method of Least Squares with Applications. Institute of Technology, Boston.

(4) BUDINOV, L. 1910 Method of mathematical treatment of data of a quantitative bacteriological analysis (Russian). *In* Viestnik Bacteriologo-Agronomical Institute of V. K. Ferein., no. 17, p. 17–29.

(5) Carvallo, E. 1912 Le calcul des probabilites et ses applications. Ganthеèr-Villars, Paris.

(6) Davenport, C. B. 1914 Statistical Methods. Wiley, New York.

(7) Davis, A. R. 1921 The variability of plants grown in water cultures. *In* Soil Sci., v. 11, p. 1-32.

(8) Fischer, H. 1909 Besitzen wir eine brauchbare Methode des bakteriologischen Bodenuntersuchung? *In* Centrbl. Bakt. (etc.), Abt. 2, v. 23, p. 144–159.

(9) Johnstone, J. 1919 The probable error of a bacteriological analysis. *In* Rpt. Lanc. Sea-Fish. Lab., no. 27, p. 64-85. (*Ref. in* Science, n. s., v. 51, 1920, p. 89–91).

(10) Mellor, J. W. 1904 Chemical Statics and Dynamics, incl. Theories of Chemical Change, Catalysis, and Explosions. Longmans, New York.

(11) Michael, E. L. 1920 Concerning application of the probable error in cases of extremely asymmetrical frequency curves. *In* Science, n. s., v. 51, p. 89–91.

(12) Waynick, D. D. 1918 Variability in soils and its significance to past and future soil investigations. I. A statistical study of nitrification in soils. Univ. Cal. Pub., Agr. Sci., 3, p. 243–270.

(13) Wood, T. B. 1911 The interpretation of experimental results. *In* Jour. Bd. Agr. (London), v. 18, (sup. no. 7) p. 15–17.

(14) Wood, T. B., and Stratton, F. J. M. 1910 The interpretation of experimental results. *In* Jour. Agr. Sci., v. 3, p. 417–440.

(15) Woodward, R. S. 1906 Probability and Theory of Errors, Wiley, New York.

DIFFERENCES EFFECTED IN THE PROTEIN CONTENT OF GRAIN BY APPLICATIONS OF NITROGEN MADE AT DIFFERENT GROWING PERIODS OF THE PLANTS

W. F. GERICKE

University of California, Agricultural Experiment Station

Received for publication September 22, 1921

In a previous paper (1), the writer has shown how the protein content of wheat (White Australian) is markedly influenced by the supply of nitrogen available to the plants at certain phases of their growth. That investigation led to further experimentation with other cereals to see to what extent the protein content of other grains might be affected. The investigation has now proceeded so far that some of the results can be given publication.

Briefly described the cultural methods employed to obtain high protein grain were as follows:

One-gallon stone jars were filled with a soil low in nitrogen which is known locally as Oakley sand. This soil as taken from the field had a low crop producing power for cereals, but it responded readily in large crop production when treated with a moderate application of $NaNO_3$ or $(NH_4)_2SO_4$. The jars filled with soil, were seeded to the following cereals:

Spring wheat (White Australian)
Oats (Texas Red)
Winter wheat (Turkey Red)
Rye (variety not known but considered a pure strain)

At the time of planting, one set of three jars of each of the different cereals received per jar, in the form of $NaNO_3$, an application of 250 mgm. of nitrogen (equivalent to 100 lbs. per acre). About two weeks later, when the plants were between 2 and 3 inches high, another set of three cultures of the four cereals named was similarly treated. At later periods other sets of three cultures of each of the four cereals received similar applications of $NaNO_3$ The complete treatment is outlined in table 1.

The essential difference in the treatments of the cultures was that of the time in the growth period of the plants when nitrogen was applied. That this was a very important factor in the physiological processes concerned in the growth of some of the cereals tested was shown by the results obtained. Differences in tillering of the plants, differences in the length of the growing period of the plants, differences in chemical composition of the grain, and differences in total dry matter production resulted from the treatments.

The plants were harvested when fully mature, and the grain was threshed, weighed and graded. Nitrogen determinations were made of samples from all cultures. This was then computed to give the protein content of the grain.

The data given in this paper deals only with the differences in the protein content of grain obtained from the sets treated with $NaNO_3$. Ammonium sulfate also was used as the source of nitrogen for other sets, but because the results obtained from these are in essence similar to the results obtained from the $NaNO_3$ treated sets, they need not be given here. The tables have been prepared to show both the days after planting and days before harvest when $NaNO_3$ was applied to the cultures. The protein content of the grain from each culture is given to show the extent of the variations obtained, and the weight of 100 kernels from each culture (excepting those of oats) gives an indication of the extent the grain was filled.

Table 2 gives the results obtained with a spring wheat (White Australian). The seed wheat used for this investigation was low in protein and high in starch,—a characteristic soft wheat. The table shows that the cultures which received the latest application of $NaNO_3$ produced wheat that had the highest protein content of all of the set. This wheat was of amber color, flinty and hard. The cultures that received $NaNO_3$ at the time of planting and 17 days thereafter, produced wheat of whitish yellow color, low in protein, high in starch—a typical soft wheat. The data show a progressive increase in the protein content of the wheat, that corresponds to each increase in the length of time after planting when nitrogen was applied, or if otherwise stated, as corresponding with each decrease in the length of the period before harvest when $NaNO_3$ was applied. This seems to be evidence that the two circumstances are related as cause and effect. It shows furthermore that a very important condition for the production of high protein wheat as obtained in this investigation is one that requires sufficient supply of available nitrogen at what appears to be certain important, perhaps, critical growth periods of the plants. This supply seemingly must be in excess of the minimum requirement needed for the formation and filling of the kernels. Table 2 shows that the protein content of the spring wheat (White Australian) is subject to a wide variation. This variation, as the investigation shows, is largely accounted for in the differences of conditions in the external environment of the plants at certain phases of their growth. The extreme differences in percentage of protein of the grain, from plants that received nitrogen at the time of planting on the one hand, and those that received nitrogen 110 days after

TABLE 1

Data showing age of spring wheat cultures when nitrogen was applied

SET NUMBER	DATE OF PLANTING	DATE OF TREATMENT	AGE OF PLANTS WHEN $NaNO_3$ WAS APPLIED	$NaNO_3$ ADDED PER CULTURE
			days	*mgm*
1	Nov. 14, 1919	Nov. 14, 1919		250
2	Nov. 14, 1919	Dec. 1, 1919	17	250
3	Nov. 14, 1919	Dec. 16, 1919	33	250
4	Nov. 14, 1919	Jan. 1, 1920	48	250
5	Nov. 14, 1919	Jan. 24, 1920	72	250
6	Nov. 14, 1919	March 2, 1920	110	250

planting on the other, and the good correlation obtained between the differences in the protein content of the grain and the corresponding treatments, is evidence that soft wheat and hard wheat, of this particular variety, is largely due to factors operative in the nutrition of the plants.

Table 3 gives the results obtained by the treatments on the protein content of winter wheat. The seed used for the tests was relatively high in protein, the grain being classed as hard wheat. The results given in this table are decidedly different from those obtained with spring wheat in that the data do not show (with the exception of that of the last treatment), that the time of application of $NaNO_3$ had any clearly definite effects upon the per cent of protein this wheat contained. That this winter wheat did not respond to the treatment as did the spring wheat, seemingly must be due to differences in the physiology of the two kinds of wheat. Winter wheat is characterized by a period of relative dormancy of growth before the plants stool or produce culms, but spring wheat is not. Under the conditions of this investigation,

TABLE 2

Effect of $NaNO_3$ *on the protein content of spring wheat (White Australin) when applied at different periods of growth*

NUMBER	DAYS AFTER PLANTING* WHEN $NaNO_3$ WAS APPLIED	DAYS BEFORE HARVEST WHEN $NaNO_3$ WAS APPLIED	WEIGHT OF 100 KERNELS	PROTEIN CONTENT	AVERAGE PROTEIN CONTENT
	days	*days*	*gm.*	*per cent*	*per cent*
1	At time of planting	201	4.57	8.9	8.9
2		201	4.27	8.6	
3		199	4.86	9.1	
4	17	183	5.11	9.6	9.2
5	17	178	5.22	9.3	
6	17	183	4.90	8.8	
7	33	162	5.11	11.3	10.6
8	33	162	5.00	10.1	
9	33	162	5.18	10.4	
10	48	147	5.22	10.7	11.4
11	48	152	5.15	11.7	
12	48	152	4.95	11.7	
13	72	135	4.92	13.1	13.0
14	72	135	4.75	13.2	
15	72	135	4.68	12.8	
16	110	121	4.00	14.7	15.2
17	110	121	4.43	15.3	
18	110	121	4 10	15 6	

* Date of planting was November 14, 1919.

spring wheat usually stools two or three weeks after the plants are up. It seems probable that the differences in the results obtained from the two classes of wheat can be partly accounted for by the relative dormancy of growth of the winter wheat. Due to this relative dormancy of growth of winter wheat the intervals of time at which $NaNO_3$ was applied to the cultures did not represent equal or comparable differences in the growth phases of winter wheat as similar intervals of time between applications of $NaNO_3$ represented different growth phases in spring wheat. Table 3 shows that only in case of the last treatment, that is when the application of $NaNO_3$ was made 109 days after planting, the grain produced was appreciably higher in protein than that produced from any of the other cultures of winter wheat. The grain obtained from the cultures that received the last application of $NaNO_3$ was approximately 25 per cent higher in protein than was that obtained from any other set of this wheat.

TABLE 3

Effect of $NaNO_3$ on the protein content of winter wheat (variety, Turkey Red) when applied at different periods of growth

NUMBER	DAYS AFTER PLANTING* WHEN $NaNO_3$ WAS APPLIED	DAYS BEFORE HARVEST WHEN $NaNO_3$ WAS APPLIED	WEIGHT OF 100 KERNELS	PROTEIN CONTENT	AVERAGE PROTEIN CONTENT
	days	*days*	*gm.*	*per cent*	*per cent*
1	At time of planting	226	3.55	14.3	14.6
2		226	3.72	15.0	
3		232	3.86	14.6	
4	21	205	3.66	14.7	13.8
5	21	205	3.97	13.0	
6	21	205	4.10	13.6	
7	36	190	3.74	14.7	14.7
8	36	179	4.09	14.0	
9	36	180	4.13	15.4	
10	60	155	3.56	13.7	13.4
11	60	166	3.13	13.7	
12	60	166	3.88	12.8	
13	81	145	3.43	13.3	14.3
14	81	152	3.51	14.5	
15	81	152	3.18	15.0	
16	109	124	3.50	17.5	17.9
17	109	124	3.00	18.6	
18	109	124	3.21	17.5	

* Date of planting was November 18, 1919.

Table 4 gives the results obtained on the protein content of oats and shows that a progressive increase resulted from the treatments. In case of the last application of nitrogen made to a set of cultures 108 days after planting, oats were produced that averaged 17.2 per cent protein, which was an increase of approximately 130 per cent over that obtained from the cultures that received nitrogen at the time of planting. The data show that the oats and the spring wheat tested exhibited certain common properties; one being that the physiological status of the plant as indicated by its growth phases is a very important factor, which affects the power and capacity of the plant to absorb and to utilize nitrogen most efficiently for the production of high protein grain.

TABLE 4

Effect of $NaNO_3$ on the protein content of oats (variety Texas Red) when applied at different periods of growth

NUMBER	DAYS AFTER PLANTING* WHEN $NaNO_3$ WAS APPLIED	DAYS BEFORE HARVEST WHEN $NaNO_3$ WAS APPLIED	WEIGHT OF 100 KERNELS	PROTEIN CONTENT	AVERAGE PROTEIN CONTENT
	days	*days*		*per cent*	*per cent*
1	At time of planting	197	Not determined	7.5	7.5
2 (Lost)		...		....	
3		197		7.5	
4	19	178		7.7	8.0
5	19	178		7.9	
6	19	178		8.5	
7	33	164		7.9	8.5
8	33	164		8.5	
9	33	164		9.0	
10	48	157		9.3	9.6
11	48	157		9.4	
12	48	157		10.0	
13	69	136		9.8	10.8
14	69	136		11.3	
15	69	136		11.2	
16	90	121		12.8	12.7
17	90	121		13.0	
18	90	121		12.3	
19	108	111		18.2	17.2
20	108	111		15.8	
21	108	111		17.5	

* Date of planting was November 6, 1919.

Table 5 gives the results obtained in the experiments with rye. The protein content of the grain of the first four sets of cultures that received $NaNO_3$

was not affected by the treatment. The grain obtained from the three last sets of cultures that received $NaNO_3$ gave a progressive increase in protein with each treatment so that the cultures which received nitrogen 133 days after planting, or 108 days before harvest, produced grain that averaged 14.0 per cent protein. This is an increase of approximately 50 per cent over that of the grain obtained from cultures that received $NaNO_3$ at the time of planting. So far as the magnitude of difference in the protein content of rye as affected by the treatment is concerned, rye occupies a place between winter wheat on one hand, and spring wheat and oats on the other. Presumably this is to be accounted for in physiology and growth habits of the plant, rye having a period of relative dormancy of growth like that of winter wheat but of shorter duration, and being unlike spring wheat and oats. The per cent of protein of the grain obtained from the first four sets of cultures was not affected by the addition of soluble nitrogen, presumably due to the fact that this

TABLE 5

Effect of $NaNO_3$ on the protein content of rye (variety unknown) when applied at different periods of growth

NUMBER	DAYS AFTER PLANTING* WHEN $NaNO_3$ WAS APPLIED	DAYS BEFORE HARVEST WHEN $NaNO_3$ WAS APPLIED	WEIGHT OF 100 KERNELS	PROTEIN CONTENT	AVERAGE PROTEIN CONTENT
	days	*days*	*gm.*	*per cent*	*per cent*
1	At time of planting	199	2.90	9.4	
2		204			9.5
3		204	3.04	9.6	
4	19	185	3.23	9.4	
5	19	185	3.16	8.7	9.0
6	19	183	3.08	8.8	
7	28	168	3.01	9.5	
8	28	168	2.81	10.5	9.7
9	28	171	2.42	9.1	
10	43	156	3.51	9.8	
11	43	156	2.80	9.6	9.8
12	43	156	2.93	10.1	
13	74	135	2.50	10.9	10.9
14	74	140	2.68	10.9	
15	105	130	3.60	11.9	12.0
16	105	130	3.27	12.1	
17	133	108	3.10	13.5	14.0
18	133	108	2.63	14.5	

* Date of planting was November 11, 1919.

nutrient was added to the plants when they were in a period of relative dormancy of growth. A correlation in the increase of the protein content of the grain and the different treatments applied after the plants had passed through the period of relative dormancy of growth seems to be proof that rye plants have a growth phase when they absorb and utilize nitrogen most efficiently for the production of high protein grain.

Inspection of the four tables show that the size of the grains as indicated by the weight per 100 kernels was not markedly affected by the treatments. Applying $NaNO_3$ early or relatively late in the growing period of the plants, did not apparently affect the extent to which the kernels were filled. The yield per culture was, as already stated, markedly influenced by the treatment. This phase of the investigation, however, will be treated in another paper.

The results of this investigation show that the chemical composition of grain such as the protein content of some of the important cereals can be markedly affected by factors involved in the nutrition of the plants. Furthermore the data show that variations in the protein content of grain are not always due to unknown genetic factors, but they may be definite non-inheritable responses of the plant to certain conditions of its external environment. It seems, from a critical analysis of the data, that in some of the nutritional processes of the plants studied are to be found certain conditions that might account for properties or characters which heretofore have been considered to be of genetic origin.

REFERENCE

(1) Gericke, W. F. 1920 On the protein content of wheat. *In* Science, v. 52, p. 446–447.

SOME RELATIONS OF ARSENIC TO PLANT GROWTH: PART 1

JOHN STEWART

Utah Agricultural Experiment Station

Received for publication September 19, 1921

INTRODUCTORY

For at least three-quarters of a century the effect of compounds of arsenic upon plant growth has been a subject of scientific investigation. It was early recognized that arsenic is quite generally found in animal tissue; and, since the animal must have obtained the arsenic from its food, it was soon found that arsenic is present in minute amounts in many plants. Later investigations (2) have shown arsenic to be very widely distributed in the soil, and to have been derived in this case from the original rocks from which the soil was formed.

The fact that arsenic was found to be so generally present in animal and vegetable tissues raised the question as to whether or not arsenic is essential to the life processes. Gautier and Clausmann (1) failed to find arsenic in *some* plants and concluded from this fact that arsenic is not essential to vegetable life. But the more intimately life processes are investigated the more apparent it is becoming that very minute quantities of substances may have a very decided effect on those processes. For example, note the decided effect which the minute amount of iodine in the thyroid gland has on animal life processes. The more recent investigations are thus making it appear that it is still too early to draw definite and final conclusions as to the rôle of arsenic in plant and animal life; and more refined methods of investigation may very probably be expected to reveal the more general presence of arsenic in plants than heretofore found.

But even though arsenic in minute quantities may or may not be useful to plant or animal life, it has been abundantly proved that it is a strong poison to both plants and animals if the quantity absorbed is large. Some years ago it was reported (5 and 6) that orchard trees were dying in Colorado as a result of arsenic poisoning. Since the practice of spraying is resulting in the application of considerable quantities of arsenical compounds to the soil and since smelters in some localities belch forth large amounts of arsenic oxides annually, it thus becomes important from a practical point of view to have more definite information as to the effects of arsenic on plants. Dr. Greaves (2, 3, 4) worked on this problem and has published some of his results in several papers. His work at first dealt with some chemical phases, but later it has dealt with the effects of arsenical compounds on the microörganisms of the soil, ammonifiers and nitrifying organisms.

In 1912 the chemical phase of this investigation was turned over to the author of this paper, who devised a plan of attacking the problem.

It was proposed to determine the solubility of lead arsenate and other compounds of arsenic, likely to be used for spraying purposes, in solutions of the inorganic salts commonly occurring in the soil, a determination being made with each pure salt. Two series of concentrations were to be used, one representing the concentrations which might be expected in an ordinarily rich soil and the other representing concentrations such as might be expected in strongly "alkali" soils. It was also proposed to determine these solubilities in mixtures of all the individual salts used in each of the two series mentioned. Some humus was to be prepared and, after removal of the ammonia, it was to be used in some more determinations with the two series of solutions to test the effect of organic matter of the soil in conjunction with the soluble salts on the solubility of the arsenic compounds. Soils were also to be collected from the principal fruit growing sections of the State. A definite quantity of each soil was to be treated with a definite volume of water and the solubility of the arsenic compounds in these mixtures was to be determined. It was also proposed to build concrete, water tight vessels, sufficiently large for the roots of a mature tree. These were to be built so the top was flush with the surface of the ground, and filled with soil the composition of which was known. Some of the orchard trees, such as apple, peach, pear, etc., were to be grown to maturity in these tanks and then subjected to the usual spraying treatment given to orchards. This treatment was to be continued for about five years or more after the tree commenced to bear, and the effects of the spraying treatment on the tree, the fruit, and the soil were to be noted. A series of tests was also to be made upon a number of young trees and shrubs grown in small vessels. For instance, about twenty to forty apple trees were to be grown in this way. These were to be divided into ten sets of two or four trees each. Each set, except one tree for control, was then to be treated with solutions containing a definite amount of sodium arsenate, each set receiving a different amount. The effect of the treatment was to be studied by means of the appropriate observations and analyses. All kinds of ordinary orchard trees were to be treated in this way. The experiment as planned was intended to be a somewhat exhaustive study of the effects and nature of arsenical poisoning on plants. Results of value from both a practical and scientific standpoint were expected. Some of the work planned has been accomplished, and this paper sets forth these results.

The methods of analysis in such work must be delicate, reliable, and as rapid and simple as possible. To select or develop the most suitable analytical methods was in itself no small task. So before commencing on the experimental part of the investigation the author made a comparative study of the methods for determining arsenic. This resulted in a decision to use the Marsh method as modified by Greaves (3) of this station and the Williamson method mentioned by Sutton (8) as modified by the author of this paper. The Williamson method is fraught with some difficulties, as a reference to the recent literature will show. The means of satisfactorily overcoming these difficulties were worked out in this laboratory. Thoroughly understood, the method becomes a rapid and excellent one, but not suitable for the determination of such minute quantities of arsenic as is the Marsh method. For further information about the analytical methods used in this work, reference must be made to the earlier paper of Greaves (3) and to the author[1] for unpublished information concerning the modified Williamson method.

---

[1] A paper by the author describing his modification of the Williamson method was accepted for publication by the *Zeitschrift fur Analytische Chemie* in the early summer of 1914, but the outbreak of the war seems to have prevented its publication.

EXPERIMENTAL

Solubility of lead arsenate in salt solutions

The modified Williamson method was used for determining the arsenic dissolved in the salt solutions and the soil solutions. The modified Marsh method was used for determining the arsenic absorbed by the plants in the pot experiment, which will be reported in another paper. The lead arsenate used for the solubility determinations was *Sherwin Williams*, guaranteed to contain not less than 25 per cent of arsenic, and not more than 1½ per cent of water soluble arsenic. It was an impalpable dry powder.

As stated above, solubility in solutions of each salt of two concentrations was determined. Potassium carbonate was used as a starting point for determining the concentrations. The weaker potassium carbonate solution contained 0.10 per cent of K_2CO_3, the stronger one contained 0.50 per cent of K_2CO_3. The concentrations of the other salt solutions were then made such that the basic elements were present in all the solutions in chemically equivalent amounts. For example, the weaker series of solutions contained in 2 liters, 2.0000 gm. of K_2CO_3, 2.1580 gm. of KCl, 1.5340 gm. of Na_2CO_3 etc.; the stronger series of solutions contained 10.0000 gm. of K_2CO_3, 10.7900 gm. KCl., 7.6700 gm. Na_2CO_3 etc. The solutions of the common salts of potassium, sodium, ammonium, calcium and magnesium prepared in this way were then treated with an excess of lead arsenate and shaken two or more times per day for three weeks or more. The solutions were filtered, one liter treated with 10 cc. of HNO_3 and 10 cc. of H_2SO_4 and evaporated to white fumes. The residue was cooled, the sides of the vessel washed down with water and again evaporated to white fumes. This dilution and re-evaporation are necessary to break down the nitrosyl sulfuric acid and thus completely remove oxides of nitrogen. The dissolved arsenic was then determined.

Some difficulties were encountered in this work and as yet no satisfactory means of entirely overcoming them has been found. Solutions such as Na_2CO_3 which give an alkaline reaction on account of hydrolysis, persistently hold the lead arsenate in colloidal solution and suspension. No way of filtering out this colloidal material was found; after the best filtration possible such solutions were still opalescent. Filtration through Chamberlain-Pasteur filters was not feasible because these retained arsenic which was in true solution. The best way found was to filter through paper. By repeating the filtration several times through the same paper the pores became somewhat clogged with the lead arsenate, and a somewhat clearer filtrate resulted. It was found by Stewart (7) that a good way to filter humus was through paper covered with a thin layer of the soil from which the humus has been extracted. The two cases are identical in principle.

The length of time required to establish equilibrium between the dissolved and undissolved lead arsenate was also an obstacle to rapid work. But even shaking the solutions containing lead arsenate for six days in a shaking machine failed to establish equilibrium so that duplicates would agree satisfactorily. Variations in temperature also result in slightly different values for the solubility.

The solubility data obtained for the various salt solutions are shown in table 1. The table is in three parts, A, B and C. Part A shows the solubility of the lead arsenate in the weaker concentrations mentioned above; part B, in the stronger concentrations; and part C, in tap water, distilled water and two mixtures of salts. Mixture 1 represents a mixture of the salts of the concentrations used in part A, and Mixture 2, a mixture of the salts of the concentrations used in part B. Some of the salts were precipitated out of solution in both mixtures. The figures in the table represent milligrams of arsenic in a liter of solution.

TABLE 1

Arsenic dissolved in the form of lead arsenate in various salt solutions

	CARBONATES	CHLORIDES	NITRATES	BICARBONATES	SULFATES	BISULFATES	PHOSPHATES	
							Secondary	Primary
A. Weaker concentrations								
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
K	165.2	39.3	4.5	139.9	4.6	241.9	174.2	29.1
Na	184.8	51.7	5.3	136.4	5.9	260.9	168.9	11.8
NH_4	175.6	37.0	3.4	136.4	3.5	271.5	177.6	18.7
Ca	73.4	54.7	2.0		5.6	296.3	73.9	36.6
Mg	4.1	36.6	3.5	37.4	4.5	268.5	93.5	21.0
B. Stronger concentrations								
K	115.9	156.0	8.1	99.8	5.8	647.2	292.9	78.0
Na	131.8	130.5	13.5	96.7	5.6	1016.9	357.0	84.6
NH_4	119.2		5.4	162.5		838.3	392.1	63.0
Ca	36.5	105.4	4.3	13.5	4.2	615.2	57.7	67.5
Mg	20.1	101.2	3.5	85.4	2.6	572.3	46.1	38.0

C. Other solvents

	p.p.m.
Tap water	33.8
Distilled water	3.0
Mixture 1	183.8
Mixture 2	324.9

In the case of sodium, potassium and ammonium carbonates and probably some of the alkali phosphates, the data represent some material that is in colloidal solution as well as that in true solution. In each case the figure in the table represents the average of two to ten determinations. It will be noted that the lead arsenate is only very slightly soluble in distilled water, 3 parts per million. In the presence of nitrates and sulfates the solubility does not appear to be very materially different from that in distilled water, there being possibly a very slight increase in solubility in the presence of these salts. Since lead sulfate is a very insoluble compound, the ionic theory would lead one to expect such a result as appears in the table in the sulfate column; but as lead nitrate is a soluble compound the ionic theory would *a priori* lead one to expect a larger solubility than appears in the nitrate column in the table. Since a great number of analyses were made from which the data in the table are condensed, there can be no doubt that lead arsenate is not materially more soluble in nitrate solutions than in pure water. The data show that the neutral chlorides increase the solubility of the lead arsenate considerably. The difference between the effect of the chlorides and the nitrates is somewhat surprising, and must apparently be attributed to the specific nature of these salts modifying the solvent. That is, chloride solutions and nitrate solutions have different solvent powers in much the same way that water and alcohol have. As is to be expected, the acid salts such as the bicarbonates, the bisulfates and the acid phosphates cause considerably larger quantities of the lead arsenate to go into solution. Increasing the concentration of the chlorides, the bisulfates and the phosphates also increases the amount of arsenic dissolved; but in the case of the sulfates and nitrates increased concentration appears to have no effect, while in the case of the carbonates and bicarbonates increased concentration as used in these experiments causes a decrease in the amount of arsenic dissolved. Ten times as much lead arsenate was dissolved in tap water as in distilled water. This, of course, shows to some extent what is to be expected to occur in the underground water. The tap water contains 212 parts of solids per million, mostly in the form of bicarbonates of calcium and magnesium.

Solubility of lead arsenate in soil solution

Soils were collected for this experiment in 1913 from the principal fruit-growing sections of the state. The amount and composition of the water-soluble material in these soils were determined. The treatment with arsenic was as follows: Two hundred fifty grams of each of the soils were treated with one liter of water. An excess of lead arsenate was added to this mixture and the amount of arsenic dissolved determined in the same manner as indicated above for the salt solutions. The data expressed in milligrams of arsenic per liter are in table 2. The soils collected represented light sandy loams, gravelly loams, rich and somewhat heavy loams, and uncultivated alkali land.

Data in table 2 have been grouped roughly according to soil types. The College Farm soil stands alone. The group of nine soils following includes in the main somewhat gravelly bench lands which are more representative of the fruit-growing districts than the other soils mentioned. Next is a group of five light sandy soils without gravel. This is a lighter type of soil than the preceding group. The fourth group includes nine good loam soils from some of the best farming lands in the State. Some of them tend to heaviness, but all are easily tillable. The last two soils in the table represent uncultivated "alkali" land west of Salt Lake City and between Tooele and Grantsville.

The data show that lead arsenate is much more soluble in the soil solution than in pure water, and that it has about the same solubility in the soil water

TABLE 2

Solubility of lead arsenate in soil solution

SOIL NUMBER	LOCALITY	SOIL TYPE	ARSENIC PER LITER	COMPOSITION OF SOIL SOLUTION						
				Total solids	Cl	CO_3 (and HCO_3)	SO_4	Ca	Mg	K
			mgm.	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
78032	College Farm	Gravelly bench loam	44.5	0.238						
78033	College Farm		43.1	0.238						
77939	East of Midvale 1 mi.	Coarse sandy loam	20.4	0.140		0.034	0.008	0.071	0.021	0.013
77942	Tooele (W. side)	Bench loam	23.8	0.214		0.050	0.014	0.084	0.059	0.014
77945	Tooele (N. E. side)		11.1	0.142		0.040	0.008	0.060	0.027	0.012
77949	S. E. of Provo	Black, gravelly loam	22.0	0.270		0.040	0.046	0.060	0.027	0.017
77950	Provo Bench (Anderson Bros. orchard)	Light gravelly loam*	16.0	0.108		0.030	0.025	0.044	0.017	0.013
77954	Orchard S. W. of Ogden		10.5	0.092		0.024	0.017	0.050	0.019	0.006
77956	Pleasant View near Hot Springs	Gravelly bench land	25.4	0.186		0.060	0.022	0.056	0.026	0.012
77962	Near S. city limits of Brigham City		11.1	0.218	0.028	0.040	0.019	0.044	0.027	0.012
77963	Near Brigham City depot		12.1	0.140		0.041	0.014	0.040	0.011	0.007
Averages..........			17.0	0.168		0.041	0.019	0.056	0.026	0.012
77951	2 mi. So. 77950	Light sandy loam	8.1	0.102		0.034	0.016	0.033	0.020	0.007
77946	Clearfield		9.6	0.146		0.022	0.012	0.036	0.027	0.008
77955	Five points		16.2	0.124		0.035	0.023	0.052	0.024	0.007
77958	North of Willard	Light loam	9.4	0.114		0.016	0.017	0.034	0.023	0.010
77960	Dewey (W. of Depot)		12.6	0.142		0.031	0.031	0.056	0.023	0.010
Averages..........			11.2	0.126		0.027	0.020	0.042	0.023	0.008

77938	West Jordan	Heavy loam	26.2	0.230		0.055	0.017	0.075	0.032	0.022
77940	North of Murray near Hussler's mill		23.1							
77944	Grantsville		31.8	0.220		0.061	0.016	0.056	0.031	0.007
77947	Farmington	Heavy black loam	19.8	0.164	0.071	0.014	0.010	0.056	0.026	0.012
77949	Centerville		14.1	0.140		0.030	0.025	0.049	0.029	0.008
77952	Provo	River bottom loam	17.0	0.210		0.060	0.024	0.066	0.022	0.011
77957	North Ogden	Heavy loam	13.3	0.180	0.014	0.050	0.017	0.056	0.024	0.014
77959	Honeyville		16.7	0.188	0.014	0.056	0.035	0.060	0.031	0.009
77961	Garland		26.0	0.412	0.028	0.048	0.043	0.080	0.025	0.070
Averages			20.9	0.218	0.032	0.048	0.023	0.062	0.027	0.019
77943	Between Tooele and Grantsville	Alkali (Uncultivated)	64.5	0.216		0.070	0.014	0.071	0.024	0.012
77941	About 1 mi. W. of S. L. C. gas plant		33.2	0.198		0.042	0.012	0.072	0.004	0.021
Averages			48.8	0.207		0.056	0.013	0.071	0.014	0.016

* Soil no. 77954 had a clay substratum.

that it has in tap water. The solubility is shown to be roughly proportional to the percentage of soluble salts in the soil. Thus the third group of soils has the lowest per cent of soluble salts and gives the lowest solubility for the lead arsenate. The second group of soils having the next higher per cent of soluble salts has the next higher solvent power for the arsenate. The fourth group stands next in order in both soluble salts and solvent power, and the College Farm soil follows the fourth group in amount of soluble salts and in solvent power for the arsenate. The two "alkali" soils in the last group break this regularity. These show a somewhat high solvent power for lead arsenate, but the soluble salts in the soils are not shown to be high. In fact, the low per cent of soluble salts found for these two soils is somewhat unexpected, but repetition of the analysis failed to change the result. It is hardly possible to trace any relation between the amount of arsenic dissolved and the individual components of the soil solution, although increase of the carbonate and bicarbonate ions and of the potassium ion is accompanied by increased solution of the arsenate. The fourth group, which contains more chlorides than the second and third, also dissolved more arsenic. These results are in keeping with the finding of Greaves (2).

SUMMARY

Lead arsenate is a very insoluble campound. Only three parts of arsenic dissolve in a million parts of pure water. Its solubility is greatly increased by many common salts when these are present in the aqueous solvent, but sulfates and nitrates do not seem to increase materially the solvent power of water for lead arsenate. Acid salts and those which hydrolize with an alkaline reaction markedly increase the solvent action of water on lead arsenate. The soil solution also has a greater solvent power for lead arsenate than has pure water.

REFERENCES

(1) Gautier, A., and Clausmann, P. 1904 Alimentary origin of arsenic in man. *In* Compt. Rend. Acad. Sci. (Paris), v. 139, no. 4, p. 101–108.

(2) Greaves, J. E. 1913 The occurrence of arsenic in soils. *In* Biochem. Bul., v. 2, no. 8, p. 519–523.

(3) Greaves, J. E. 1913 Some factors influencing the quantitative determination of arsenic in soils. *In* Jour. Amer. Chem. Soc., v. 35, no. 2, p. 150–156.

(4) Greaves, J. E. 1913 Some factors influencing ammonification and nitrification of soils. I. Influence of arsenic. *In* Centbl. Bakt. (etc.), Abt. 2, v. 39, no. 20, 22, p. 542–560.

(5) Headden, W. P. 1908 Arsenical poisoning of fruit trees. Col. Agr. Exp. Sta. Bul. 131.

(6) Headden, W. P. 1910 Arsenical poisoning of fruit trees. Col. Agr. Exp. Sta. Bul. 157.

(7) Stewart, John 1911 Original communications. *In* Proc. Eighth Internat. Cong. Appl. Chem., v. 15, p. 226.

(8) Sutton, Francis 1904 A Systematic Handbook of Volumetric Analysis, 9th ed. P. Blakiston's Sons & Co., Philadelphia.

SOME RELATIONS OF ARSENIC TO PLANT GROWTH: PART 2

JOHN STEWART AND EDWIN S. SMITH

Utah Agricultural Experiment Station.

Received for publication September 19, 1921

A POT EXPERIMENT

The general plan of the investigation of which this paper reports a part was outlined in Part I by the senior author. This paper reports data obtained in one of the small-scale pot experiments carried out during the summer of 1914.

Description of the experiment

Five species of plants were used in this experiment, viz., pea, radish, wheat, potato and bean. The soil used was a gravelly bench loam taken from the College Farm. The gravel was removed by sifting. The water-soluble material in this soil amounted to 0.216 per cent. Into each of the 3 gallon, glazed, stoneware jars used in the experiment, a definite weight of soil was placed. This varied from 7000 to 9000 gm. After the crop had been planted, a mulch of 500 gm. of sand was added to each jar. The moisture in the soil used was determined, so the actual dry weight of the soil in each jar was known.

Ten jars were used for each plant studied. These were divided into five sets of two jars each. One set to which no arsenic was added was used for the control plants. During the course of the experiment disodium arsenate was added to the other four sets in varying amounts. Each of the five kinds of plants received the same treatment.

There was some delay in getting the experiment started so the planting did not occur until the middle of June. In some cases the plants did not come up well which necessitated replanting at a later date. This was the case with the wheat, and one or more of the pots had to be replanted for each kind of plant. In particular the radishes were planted very late in the season. This was because lettuce which had been planted two or three times in these pots had failed to grow. Radishes were then substituted for this crop. Some of the potatoes failed to grow at all, and on account of the lateness of the season attempts to get plants in all the potato pots were abandoned. None of the plants, therefore, had arrived at maturity when the experiment was closed on September 30.

TABLE 1

Weight of plants produced and parts of arsenic absorbed per million parts of dry plant

POT NUMBER	APPEARANCE ABOVE GROUND	ARSENIC TREATMENT		AVERAGE AMOUNT OF ARSENIC ABSORBED BY EACH PLANT ON BASIS OF DRY WEIGHT	TOTAL WEIGHT OF DRY PLANTS	AVERAGE WEIGHT PER PLANT
		Commenced	Amount added on basis of dry soil			
Peas						
			p. p. m.	*p. p. m.*	*gm.*	*gm.*
1 and 2	July 30		0.0	0.0	18.2116	1.1318
3 and 4	30	Aug. 19	25.0	98.0	14.8257	1.4826
5 and 6	30	19	75.0	193.0	16.2201	0.9541
7 and 8	30	19	250.0	1190.0	4.0407	0.8081
9 and 10	*	*	500.0	2150.0	1.7503	0.1591
Radishes						
11 and 12	Aug. 18		0.0	0.0	22.8076	....
13 and 14	18	Sept. 2	25.0	53.0	26.0506	....
15 and 16	18	2	75.0	148.0	30.3656	....
17 and 18	18	2	150.0	448.0	25.4080	....
19 and 20	18	2	500.0	940.0	19.6671	....
Wheat						
21 and 22	July 25		0.0	0.0	18.867	0.5391
23 and 24	25	Aug. 19	25.0	21.0	22.8928	0.7894
25 and 26	25	19	75.0	52.0	19.7736	0.3954
27 and 28	25	19	250.0	269.0	12.1449	0.4048
29 and 30	25	19	500.0	....	not weighed	not weighed
Potatoes						
31 and 32	June 24		0.0	0.0	9.1891	9.1819
33 and 34	25	Aug. 19	25.0	78.0	27.7595	6.9399
35 and 36	24	19	75.0	347.0	3.2245	1.6122
37 and 38	28	19	250.0	524.0	7.9895	7.9895
39 and 40	crop failed					
Beans						
41 and 42	July 11	Aug. 19	0.0	Trace	14.4565	2.8911
43 and 44	28	19	25.0	50.0	11.9991	1.9998
45 and 46	26	19	75.0	155.0	7.9218	1.1317
47 and 48	26	19	250.0	678.0	7.2346	1.8086
49 and 50	19	19	500.0	1965.0	2.3626	0.4725

* Plants appeared above ground Aug. 24 in pot 9 and July 30 in pot 10; arsenic was added to pot 9 on Sept. 2, to pot 10 on Aug. 19.

Since the age of the plant might affect its power to withstand the poison, it was intended to allow the plant to get a pretty substantial growth before the treatment with arsenic was commenced. The plants were, therefore, allowed to grow as long as possible before adding any arsenic to the soil. The length of time intervening between the appearance of the plants above the soil and commencement of the arsenical treatment varied from three to six weeks, the dates in each case are given in the table.

During the experiment the moisture content of the soil was kept at approximately 20 per cent, by adding water at the surface three times a week. When the treatment with arsenical solution commenced the disodium arsenate solution was added with the irrigation water, in nine equal dosages. It required, therefore, three weeks to add all the arsenic used in the tests. In those cases in which the arsenical treatment commenced on August 19 the arsenic was all added by September 8. In these cases, therefore, a growing period of three weeks intervened between the end of the arsenical treatment and the end of the experiment.

At the close of the experiment of the plants, including roots, stems and foliage were harvested. They were dried, weighed and analyzed for arsenic. In making the analyses, all the plants produced in one jar were taken together. The adhering particles of soil were washed from the roots with distilled water, so the arsenic found in the analysis was what had actually been absorbed by the plants. The method of destroying organic matter and obtaining the arsenic in solution used in this work is described by Fresenius (3). The Marsh method, as modified by Greaves (5), was used for determining the arsenic. Results are given in table 1.

Discussion of data

The conclusions to be drawn from a preliminary and brief experiment of this kind are mainly of a tentative character, except possibly as the results may serve to corroborate earlier observations. The fact that arsenical compounds are poisonous to plants has been established, and this experiment corroborates that conclusion. But there is not a great deal of evidence in the literature showing that higher plants are stimulated to more vigorous growth by arsenical compounds.

At Rothamsted, water cultures by Brenchley (1) in very dilute sodium arsenate solutions 0.004 part per million as the lower limit), containing the essential nutrients, failed to show any stimulation of peas or barley. However, at that station, barley grown in nutrient solutions containing 1.0 to 0.05 part of arsenious acid per million looked as if stimulation had occurred, but the dry weights of the plants did not support that conclusion. Knop (7), in 1884, found that 50 parts of arsenic acid per million of nutrient solution did not check the growth of a strongly rooted maize plant which was transferred to the solution containing arsenic acid. There seems to have been very few experiments made up to date to show the effect of arsenic compounds on plants when grown in their natural habitat, the soil. Statements made by some of the earlier investigators in this line seem somewhat remarkable in the light of present knowledge. For instance, Davy (2), in 1859, and Gorup-Besanez (4), in 1863, agree in the statement that arsenious acid is without effect on peas grown in the soil. But at Rothamsted (1) peas were found to be rather sensitive to arsenical poisoning; and we have found, as herein reported, that peas are quite sensitive to the presence of disodium arsenate in the soil. Disodium arsenate is now well known to be a less energetic form of the poison than the arsenious acid used by Davy and Gorup-Besanez.

So the literature at the present time indicates that it is very doubtful whether or not compounds of arsenic are able to stimulate the growth of the higher plants, although it is now well known that certain molds and other lower plants are stimulated by, and grow luxuriantly in the presence of large quantities of arsenic compounds. Greaves (6) reported a stimulation of nitrifiers and ammonifiers in the presence of several arsenic compounds.

It seems reasonable to expect to find such a stimulation in the higher plants, but what are we to use as a criterion of such stimulation? If stimulation occurs will the dry weight of the plant necessarily be greater than it otherwise would have been? Is not a healthy, vigorous appearance also evidence of beneficial influence? The life processes of a healthy looking plant may proceed more rapidly than those of a plant of less vigorous appearance, and the former may arrive at maturity at a somewhat earlier date than the latter without attaining a greater weight.

Our experiment seems to indicate that disodium arsenate in the lower concentrations had a beneficial influence on the plants in all the cases. So far as appearances can be relied on, beans, potatoes, peas and wheat seemed more vigorous and healthy in the pots containing 25 parts of arsenic per million of soil than they did in the control pots, and in the case of peas and wheat the plants in the pots containing 75 parts of arsenic also seemed to be stimulated. this healthy appearance in the case of wheat especially, and to a less degree in the case of beans, is shown in plate 1. The photographs show the effect of the arsenic in producing apparent stimulation in the lower concentrations and checking growth and causing death in the higher concentrations. The dry weights of these plants, as shown in table 1, however, do not give definite evidence of having been increased by the arsenic in the lower concentrations, although they show plainly a decrease for the higher concentrations. Unfortunately, the value of the dry weight data is not as great as it would have been if there had been the same number of plants in each pot.

The radishes, on the other hand, gave no visible evidence in the foliage of the effect of the arsenic. But when the crop was harvested at the end of the experiment the parts of the plants below ground showed plainly the effects of the different treatments. The radishes grown in the pots of low arsenic content had thick fleshy, fine looking roots, while those grown in the pots of high arsenic content were much longer and of smaller diameter. In this case the dry weights shown in table 1 seem to indicate rather decidedly that the arsenic had had a stimulating influence up to and including the concentration of 250 parts of arsenic per million of soil. The radish plants were not counted, but the stand seemed very uniform in all the pots.

Our data indicate that the plants used are not equally resistant to the effect of the arsenical poisoning. This accords with the observations of others

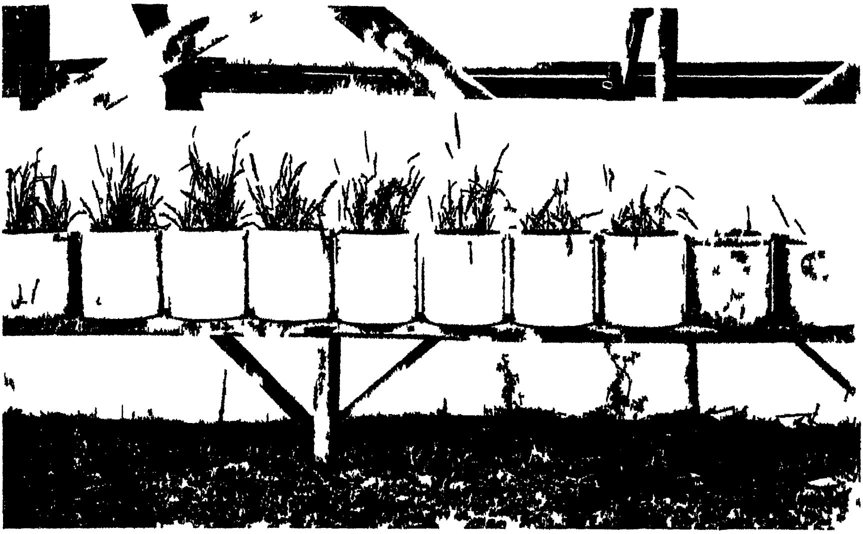


Fig 1 Wheat Growing on Soil Treated with 0 to 500 Parts of Soluble Arsenic per Million Parts of Dry Soil

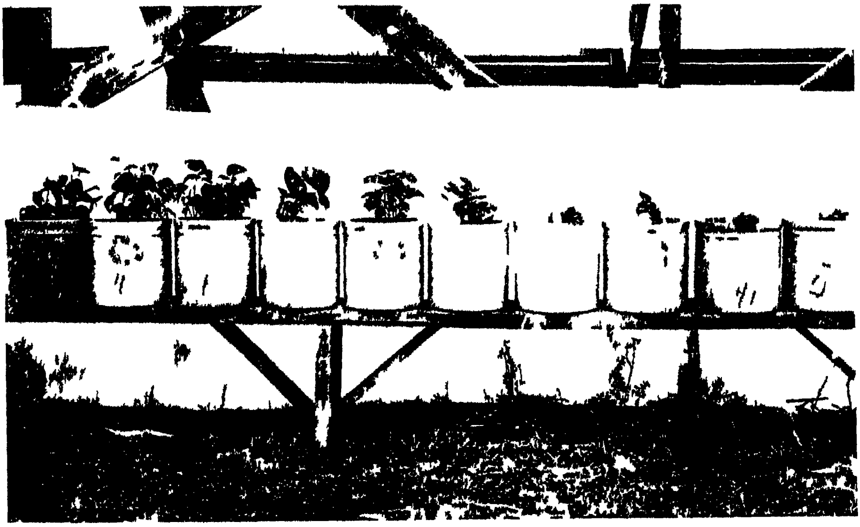


Fig 2 Beans Growing on Soil Treated with 0 to 500 Parts of Soluble Arsenic per Million Parts of Dry Soil

to the effect that the specific nature of the plant is an important factor in its behavior toward poisons. Beans seemed decidedly more sensitive to the poison than the other plants. Those in pots 49 and 50 were dead on August 25 after only two treatments with arsenic. On September 8 at the close of the arsenical treatment the beans in pots 47 and 48 were turning yellow and appeared very sick, while those in pots 45 and 46 had some yellow leaves. Beans of good size were produced by the plants in pots 41 to 44, inclusive, but in pots 45 and 46 only small, immature beans were produced. Potatoes seemed a close second to beans in sensitiveness. The plant in pot 37 was a large healthy one before treatment with arsenic, but it soon showed the yellow withering of the lower leaves. The plants in pots 33 and 34 were the only ones that produced tubers, and these measured 1 to ½ inch in diameter. Peas were more resistant than beans and potatoes and seemed more sensitive than wheat. In pots 9 and 10 the peas commenced to wilt and turn yellow in the lower leaves after three treatments with arsenic. They died after six treatments. Radishes seemed decidedly more resistant than the other plants tested, although those in pot 20 died after five arsenical treatments. Still, those in pot 19 undergoing duplicate treatment flourished and made a better yield than either of the control pots, 11 or 12.

The amount of arsenic absorbed by the plant which is necessary for the checking of growth seems to have been, in parts per million of dry plant matter, 50 for beans, 78 for potatoes, 52 for wheat, 193 for peas, and 940 for radishes. When the plant is killed the smallest amount of arsenic absorbed was shown to be 269 parts per million for wheat, 524 for potatoes, 678 for beans, 1190 for peas, 940 for radishes.

The wheat was not killed entirely even in the pots containing the most arsenic, 500 parts per million parts of dry soil, and, in the two pots containing 250 parts per million, the plants made a decided recovery after the arsenical treatment had ended on Sept. 8. While the wheat seemed more resistant to the arsenic in the soil than did the other plants except radishes, it had absorbed in each case less arsenic in proportion to its dry weight. Its apparently greater power of resistance may thus be due to a difference in root activity rather than to the power of the plant to live with more or less arsenic in its tissues. We were unable to determine the arsenic actually absorbed by the wheat in the pots treated with 500 parts of arsenic per million of soil on account of the fact that the dead parts of the plants had become soaked with the unabsorbed arsenic in the soil during a rainstorm. This is the reason also for the high amount of arsenic reported in the radishes in pot no. 20. The value of the data showing the amount of arsenic absorbed by the wheat and the weight of the wheat plants is, however, somewhat marred, though not completely destroyed by the fact that a horse grazing on the campus ate off the tops of all the wheat plants after September 25th.

Is the fine, healthy appearance of plants grown in the presence of small quantities of arsenic compounds due to the destruction, by the arsenic, of soil microörganisms which are injurious to the higher plants or is it due to a direct action of the arsenic compound on the higher plant? While in water cultures injurious microörganisms are not necessarily excluded, still the fact that this healthy appearance of the plants develops in these cultures tends to show that it is due to a direct chemical-physical action. But both kinds of action may be involved. The problem is interesting and awaiting solution.

In our experiment, as in those of others already reported, and in the literature, the effect of arsenical poisoning of higher plants is seen to be a destruction of the chlorophyll with accompanying death of the leaves affected. This yellowing of the leaves commences at the lower ones and gradually extends up the plant. This indicates that the poisoning is probably due to a chemical action on the chlorophyll in which the arsenic is involved. If the poisonous action were due in the main to injury to the root or tissues of the stem, its visible effects should be first seen at the top of the plant and extreme tips of the leaves, which is exactly the opposite of what is actually observed.

In this experiment it appears that 75 parts of arsenic per million of soil is not injurious to some plants, while the more sensitive ones are slightly affected, but 25 parts appear to be stimulating. These quantities are equivalent to about 375 and 125 parts per million of soil solution. In the previous paper (Part 1) by the senior author, it was shown that lead arsenate is soluble in the soil solution to the extent of about 10 to 64 parts of arsenic per million of soil solution. These results indicate tentatively, at least, that spraying orchards with lead arsenate may safely be continued for a number of years from the beginning of the spraying.

SUMMARY

Our results give visible evidence of stimulation, or beneficial influence, of disodium arsenate in low concentration on all the plants tested. In the case of the radish, however, this visible result appears in the underground part and not in the foliage. The evidence of stimulation or non-stimulation shown by the dry weights is inconclusive, but indicative of stimulation where the concentration of the arsenate was low, and in the case of the radish in all the concentrations tested. It would, therefore, seem that the accumulation of arsenic in the soil, as a result of the spraying of orchards, if not continued to excess, may be beneficial rather than injurious. A thorough study of the questions involved herein would probably lead to interesting and important results and conclusions in relation to the problems of disease resistant plants and of immunity in general; and, since the chlorophyll is involved in the poisoning, the problem evidently includes the very fundamentals of plant growth.

REFERENCES

(1) Brenchley, Winifred E. 1914 Inorganic Plant Poisons and Stimulants. Cambridge Agricultural Monograph, The University Press, Cambridge.

(2) Davy, E. 1859 Presence of arsenic in plants cultivated for food. *In* Gard. Chron., Sept. 10, p. 739–40.

(3) Fresenius, C. Remigins 1906 Quantitative Chemical Analysis (trans. by Alfred I. Cohn), v. 2, p. 696. John Wiley & Sons, New York.

(4) Gorup-Besanez, E. von 1863 Über das Verhalten der vegetirenden Pflanze der Ackererde gegen Metalgifte. *In* Ann. Chem. Pharm., v. 127, p. 243–256.

(5) Greaves, J. E. 1913 Some factors influencing the quantitative determination of arsenic in soils. *In* Jour. Amer. Chem. Soc., v. 35, no. 2, p. 150 156.

(6) Greaves, J. E. 1913 The influence of arsenic upon the biological transformation of nitrogen in soils. *In* Biochem. Bul., v. 3, no. 9, p. 2 16.

(7) Knop, W. von 1884 Über die Aufnahme verschiedener Substanzen durch die Pflanze welche nicht zu den Nährstoffen gehören. *In* Jahresber. Agr. Chem. v. 7, neue Folge, p. 138–140.

NITROGEN FIXATION IN ARID CLIMATES

B. H. WILSDON AND BARKAT ALI

Punjab Agricultural College

Received for publication October 18, 1921

In a country such as the Punjab, the soil receives very small applications of manure. It is estimated that in a typical irrigated colony tract, the land receives on the average not more than between one-half to one ton of farm yard manure each year. In non-irrigated tracts (so called *barani* land) it may be taken that no manure is added to the soil since any manure available is applied to well irrigated land in the neighborhood. In some tracts it has been the custom to grow wheat after wheat with no application of manure at all. The economic conditions and the conservatism of the peasantry make any general use of commercial organic manures, such as cake, a remote possibility for a long time to come. Any possibility of bringing under control the factors which determine the natural processes of nitrogen fixation which must be taking place in the soil, is therefore of enormous importance.

That the natural recuperative powers of soils in arid regions must be considerably greater than those met with in more temperate climates, is shown by crop yields obtained when moisture conditions are favorable. Yields of wheat, which would certainly not be expected from a similar soil in a temperate region, are obtained on what would be regarded, from its history, as totally exhausted land. It would seem justifiable to suppose therefore that the climate may have a large influence on the activity of free-living nitrogen-fixing organisms. Moreover, it is possible that the frequent cultivation which the tradition of the peasant leads him to give his soils during the hot-weather fallow between his wheat crops, may not only conserve moisture but also bring about suitable conditions for active nitrogen fixation.

There can be no doubt that in *barani* tracts agriculture is almost wholly dependent on the natural processes of nitrogen fixation; that these processes are of almost equal importance under the better conditions of agriculture possible in irrigated tracts may be seen from the following considerations. Table 1 gives the area under various crops in the Lower Chenaab Canal colony, together with a conservative estimate of their average yield. The amount of nitrogen in the total produce is then calculated, largely on the basis of the analyses of Sen (1). This figure shows, on comparison with the average application of farm yard manure, that artificial sources can only account at most for about one-sixth of the total produce. Otherwise expressed, natural processes of fixation add something in the neighborhood of 38 pounds of combined nitrogen per acre per year.

TABLE 1

Estimate of average annual loss of nitrogen by cropping

CROP	AREA SOWN	YIELD PER ACRE		TOTAL NITROGEN CONTENT		TOTAL NITROGEN CONTENT OF CROP	
		Grain	Straw	Grain	Straw	Grain	Straw
	acres	*maunds**	*maunds*	*per cent*	*per cent*	*maunds*	*maunds*
Wheat	986,591	16	35	1.50	0.5	236,800	172,700
Barley	17,751	12	20	1.49	0.6	3,175	2,130
Rice	32,075	20	30	1.01	0.6	6,480	5,774
Maize	103,197	22	60	1.34	2.0	30,420	123,836
Mixed grain	10,509	23	35	2.68	0.7	6,479	2,575
Great Millet (*Jowar*)	16,071	8	60	1.40	0.6	1,800	5,785
Spiked Millet (*Bajra*)	35,092	9	60	1.86	4.7	5,874	98,960
Italian Millet (*Kangni*)	377	8	20	1.91	1.8	57	135
Gram	55,578	12	30	3.06	0.7	20,490	11,671
Lentils, etc.	6,317	8	15	3.94	0.8	1,991	748
Cotton	315,067	5	60	2.82	0.5	44,430	94,520
Til	777	10	30	3.62	0.5	281	116
Sarson, Toria (*Brassica Compestris*)	236,045	7	25	3.30	2.6	54,540	135,400
Linseed	1,200	6	8	3.19	0.5	229	48
Sugar Cane	59,443	300		0.19		33,890	
Fodder	378,607	200		0.8 (Dry)		181,731	
Totals	2.258.328					633.667	672.407

	maunds
Total nitrogen removed	1,306,074
Nitrogen added as manure, estimated at 21 maunds of farm yard manure per acre analysing at 0.475 per cent nitrogen	225,268
Difference equivalent to about 38 lbs. nitrogen per acre	1,080,806

* One maund = about 82 lbs.

TABLE 2

Nitrogen per 100 grams in lyallpur soils on dates shown

DATE OF SAMPLING	SANDY LOAM	LOAM	CLAY LOAM
	mgm.	*mgm.*	*mgm.*
May 15, 1916	22.8	39.4	51.0
June 13, 1916	31.0	41.0	62.0
July 1, 1916	29.0	48.0	54.0
July 17, 1916	29.0	48.0	56.0
August 15, 1916	25.0	41.0	49.0
September 18, 1916	39.0	53.0	67.0
December 1, 1916	127.0	83.0	97.0
Land prepared and wheat sown			
March 14, 1917	70.0	84.0	69.0
Wheat growing; harvesting to be done after a month			

In the year 1916 an extended series of field analyses were initiated at Lyallpur by the late J. H. Barnes (2). These were continued and supplemented with laboratory investigations by the present authors in succeeding years. In 1916 most remarkable results were obtained. The average fixation in four different districts of the Punjab amounted on the average to more than 100 per cent of the total nitrogen in the soil. The possibility of any consistent error is precluded by the results of a more detailed examination of the soils of Lyallpur where the nitrogen content of the soil was estimated at frequent intervals between the wheat harvest in April and the date of sowing in the following November. The results are reproduced in table 2 and plotted together with the rainfall in figure 1. It will be seen that with all three soils examined, after a preliminary period of depression during the rains, rapid increase took place during September and October. The astounding magnitude of the fixation may be realized by considering that an addition of 50 mgm. of nitrogen per 100 gm. of soil (the average fixation in 1916) corresponds with an application of about 150 tons of farmyard manure to the acre.

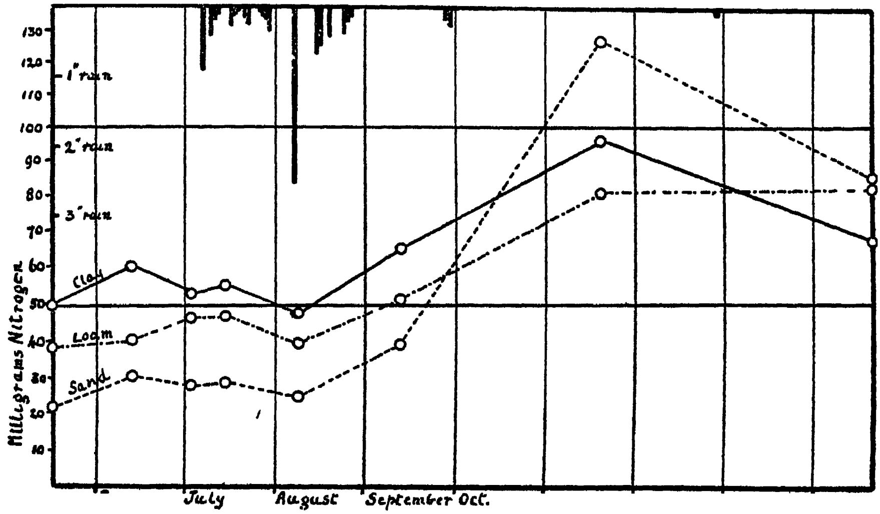


FIG. 1. RELATION OF RAINFALL AND FLUCTUATIONS IN NITROGEN CONTENT OF LYALLPUR SOILS

In 1917 experiments were continued on a smaller scale but failed to indicate any fixation. A more extended series of analyses were made in succeeding years, great attention being paid to accuracy of sampling, and the limits of the experimental error. The results will be found in detail in the Report of the Agricultural Chemist, (loc. cit.) for 1918 and succeeding years, and can only be summarized here. As a result of a calculation of the probable field and laboratory errors it was decided to disregard as evidence of either fixation or denitrification any changes smaller than 8 per cent of the total nitrogen content of the soil. The results of the large number of observations made are difficult to summarize concisely. The results are also extremely variable on account of differences of climate and cultivation. In table 3 the soils have been divided into two classes, those in which the rainfall is above 10 inches and below 10 inches. These classes are again subdivided according to the treatment received by the soil. It will be seen that in no case during the succeeding years do the results approach in magnitude those obtained in 1916. Although isolated instances of considerable fixations occur, there is no uniformity. It is also impossible to correlate the detailed results with either rainfall, cultural, or temperature factors. By the collection of more evidence this may ultimately become possible; for the present we must recognize that peculiarly favorable conditions must have prevailed in 1916. One cannot help comparing the sudden activity of the nitrogen-fixing organisms in the soil of the Punjab with the increase of virulence of pathogenic organisms as in the case of the influenza epidemic of 1919. We are at present equally ignorant of the predisposing causes in both these cases.

In order to gain some insight into the factors controlling fixation in the field, in 1919 an elaborate series of laboratory experiments was started. The following hypotheses may be entertained:

1. It may be assumed that if the great heat of the Punjab summer causes partial sterilization of the soil, when brought under optimum conditions, azotabacter will have a better chance to develop rapidly in the absence of predatory protozoa.
2. Azotobacter alone may not be the determining factor; suitable conditions may be requisite for the development of another agent either previously to, or together with, the development of azotobacter. Thus it is well known that the presence of carbohydrate food is necessary for the functioning of azotobacter, and it has been suggested that a symbiotic relationship exists between it and species of algae which develop in the soil.

In order to test these hypotheses, samples were taken at various dates throughout the fallow, which were then brought under, as far as possible, optimum conditions in the laboratory and the rate of change of nitrogen content determined. In order to test the possibility of partial sterilization in the field, the plots from which samples of the two types of soil examined were taken were duplicated, one receiving normal fallow treatment (cultivation after rain), the other being kept repeatedly stirred. The samples thus taken were brought to their optimum moisture content and incubated under three separate conditions until the following November. A sufficient number of separate samples of the main samples taken each month were set aside in order to provide the necessary duplicates for analysis each succeeding month. Of the three series of samples, one was incubated in the laboratory in diffused light, and another in a dark incubator. It was thought that any differences observed in the rate of nitrogen fixation in these two series might be due to the fact that algae would be unable to develop in the dark. The third series was placed in large earthenware pots sunk flush with the ground out of doors, but protected from rain and dust by glass plates.

TABLE 3

Abstract of nitrogen-fixation results, expressed as percentages of original nitrogen contents of soil, and classified according to year, rainfall, cultivation

(Figures with a negative sign prefixed represent losses in total nitrogen)

CLASSIFICATION OF SOILS	1916				1917				1918				1919				1920			
	Number of observations	Fixation of nitrogen			Number of observations	Fixation of nitrogen			Number of observations	Fixation of nitrogen			Number of observations	Fixation of nitrogen			Number of observations	Fixation of nitrogen		
		Highest	Lowest	Average		Highest	Lowest	Average		Highest	Lowest	Average		Highest	Lowest	Average		Highest	Lowest	Average
		per cent	*per cent*	*per cent*		*per cent*	*per cent*	*per cent*		*per cent*	*per cent*	*per cent*		*per cent*	*per cent*	*per cent*		*per cent*	*per cent*	*per cent*
Rainfall, 0–10 inches																				
Cultivated	3	413.0	90.2	204.7	3	−36.4	−4.5	−23.2	22	21.4	−12.9	−0.3	10	9.7	−12.4	−0.5	6	8.7	−14.2	−2.8
Uncultivated													9	10.7	−8.6	0.7	5	9.5	−11.6	−1.1
Rainfall, 10–15 inches																				
Cultivated	6	199.0	66.7	132.4					9	30.9	−21.8	*±0.0	6	3.4	−5.7	−1.1	1		−1.7	−1.7
Uncultivated													7	13.1	−11.9	+0.2				

* Would seem to be 4.5 per cent.—Ed.

The most direct method of testing the hypotheses would no doubt be to supplement experiments similar to those described above by enumeration of the number of azotobacter, protozoa and algae. The technical difficulties involved are however very great, so it was decided in the preliminary experiments to rely solely on the chemical evidence. Qualitative results were obtained as to the prevalence of protozoa, but the evidence was not sufficiently reliable to enable any conclusions to be based on it. It is hoped that this aspect of the work will be developed in the future.

The largest fixation observed was not more than 45 per cent, showing that we had not been successful in reproducing the favorable conditions of 1916. The results were, moreover, very variable, due probably to the difficulty of keeping the soil of small samples at optimum moisture content for long periods and at the same time avoiding spoiling the texture. It is possible however to draw certain preliminary conclusions. The most marked and uniform fixation with all soils and under all conditions of incubation took place in September. This is the period at which the rapid increase took place in the field in 1916. Thus of 12 samples the average fixation was 15.5 per cent, and with one soil about 40 per cent. At no other date was there a consistent fixation with all samples. With the samples isolated in May and incubated till the following October, no definite increase was observed which was far outside the limits of the allowable error. The general course of events however appears to be similar, both with the different soils, and different conditions of incubation, as will be seen from table 4.

It seems legitimate to conclude, therefore, that the date of sampling is of the utmost importance in estimating the nitrogen-fixing powers of a soil in the laboratory. There appears to be a definite seasonal influence which must be taken into account.

The results so far obtained fail to enable us to form an opinion as to the effect of the partial sterilization possible in the soil under ordinary cultural conditions. In some cases the fixation was greater in the soil taken from the pulverized plots, and sometimes the reverse. The experiment was however conclusive in showing that it is not till after a prolonged period of dry heat that the soil becomes capable of considerable nitrogen fixation. Much more data must be made available before we can hope successfully to correlate the results of chemical and biological examination with the seasonal influences. Our present knowledge appears to indicate that it is the seasonal influence which is of primary importance. It may therefore be necessary to await the passage of many seasons before it will be possible to discover all the factors which control nitrogen fixation in the soil. As in the case of influenza, the endemic activities of the soil organisms may afford much valuable information but it may be necessary to wait for the next epidemic before we shall be able to solve the problem of their sudden virulence.

Another aspect of the question which remains to be studied is seen if we consider that in order to utilize the nitrogen fixed in the soil, it must be subsequently nitrified. From the results obtained it appears that a rapid period of nitrogen fixation is followed by an almost equally rapid loss. If, however, it is found possible to control the nitrification of only a small fraction of the amounts of nitrogenous organic material which may be synthesized in the soil, we shall have travelled a long way in the direction of making the soil self-supporting in its nitrogen economy.

Almost the whole of the laboratory work referred to in this paper has been carried out by Mr. Barkat Ali. Acknowledgment must also be made to Mr. S. M. Nasir for much painstaking assistance.

REFERENCES

(1) SEN, J. N. 1917 The composition of some Indian feeding stuffs. Pusa Agr. Res. Inst. Bul. 70.

(2) WILSDON, B. H. 1920 Nitrogen fixation in Punjab soils. Ann. Rept. Agr. Dept., Punjab, pt. 2, p. 27–30.

OXIDATION OF IRON PYRITES BY SULFUR-OXIDIZING ORGANISMS AND THEIR USE FOR MAKING MINERAL PHOSPHATES AVAILABLE[1]

W. RUDOLFS[2]

New Jersey Agricultural Experiment Stations

Received for publication October 18, 1921

INTRODUCTION

It is very well known among sulfuric acid manufacturers and others that iron sulfides (pyrites) tend to oxidize slowly into sulfates. These changes seem to be more rapid when the pyrites are moist or lying on the gound. They first oxidize, forming ferrous sulfate, and since this compound is not stable in presence of water or moist air, this is converted into basic iron sulfates and then into iron hyroxides which are but slightly soluble.

Löhnis (3) describing the influence of microörganisms on the sulfur cycle (p. 705) says that they do the principal work. The sulfides are transformed into hydrogen sulfide; the same may happen with the thiosulfates and more seldom with the sulfates. The H_2S and the thiosulfate are oxidized into sulfates. At another place, however, (p. 708) he lays emphasis on the fact that the oxygen of the air has a strong oxidizing action. Allen and Johnston (1) found that when pyrites are ground for analyses they suffer partial oxidation to sulfur dioxide and ferrous sulfate. Whether or not this action is of a purely chemical nature Kappen and Quensell (5) have tried to ascertain. They weighed into a porcelain dish, in the bottom of which was a filter paper, 100 grams of sandy soil, moistened with 15 cc. of water, and drained off the surplus with a suction pump. Hydrogen sulfide was passed through the soil till the mass had a black color. The soil was then placed in the open air and in 30 minutes the color had changed and the iron sulfide changed into iron sulfates. After standing for 5 hours the soil was treated with 10 per cent HCl and the amount of sulfuric acid determined in the extract. For 100 grams of untreated soil they found as an average 2.11 mgm. of sulfur as sulfates at the beginning and 2.34 mgm. after 5 hours. With the treated soil they found 4.15 mgm. at the beginning and 5.01 mgm. after 5 hours. They draw the conclusion: "dass überall da, wo sich im boden durch Faulnis von Eiweisstoffen oder durch Reduktion von Sulfaten Schwefelwasserstoff bildet, oder sofort durch dass wohl in allen Böden in dazu genügenden Mengen vorhandene Eisenoxydhydrat unter Schwefelabscheidung und Reduktion des Eisenoxydes gebunden wird, und dass das Schwefeleisen, die Zutrittsmöglichkeit von Sauerstoff natürlich vorausgesetzt, sich ohne Einwirkung von Mikroorganismen weiterzersetzt. Die höchste Oxydationsstoffe des Schwefels, die wieder ohne weiteres in den Kreislauf eintreten könnte, entsteht hierbei nicht, oder nur in spuren; es bildet sich vielmehr hierbei fast ausschiesslich elementarer Schwefel." Their further studies led them to conclude that this elementary sulfur is changed to sulfates by various influences. Van Bemmelen (7, p. 81, 85, 97, 98) pointed out that pyrites occur locally in diatoms and also in plant cells. He explains the formation of pyrites as a sulfate reduction. The iron in the soil is changed into iron sulfates in the presence of sulfates. By the oxidation of the iron sulfite sulfuric acid is formed. This sulfuric acid does not attack, or but very slightly attacks the silicates in the soil, but does attack the iron oxide present. This investigator concludes (p. 97) "that the iron oxide in the soil protects, to a certain extent, the humate and silicate bases against the action of the sulfuric acid." Iron sulfate is formed from this acid and the iron after the oxidation of the sulfur to sulfuric acid.

---

[1] Paper No. 91 of the Journal Series, New Jersey Agricultural Experiment Stations, Department of Soil Chemistry and Bacteriology. This paper will appear in Rutgers College Studies, Vol. 1.

[2] Part of a thesis submitted to the faculty of Rutgers College and the State University of New Jersey in partial fulfillment of the requirements for the degree of Doctor of Philosophy. This work was started in France and partially repeated at the New Jersey Agricultural Experiment Stations.

The studies made by the writer upon this subject are in general accordance with Van Bemmelen's conclusions, but so far as biological factors are concerned, considerable room for study is left.

PURPOSE OF INVESTIGATION

From the work of Kappen and Quensel, Allen and Johnston and others it would seem that the action of bacteria on the transformation of iron sulfide into iron sulfate is slight or negligible.

Two main points were open for investigation:

A. Do sulfur oxidizing organisms transform iron sulfide into iron sulfate?

B. If so, what is the influence of this transformation on the availability of rock phosphate composted with a mixture of pyrites, sulfur and soil?

EXPERIMENTAL RESULTS

Series 1

A slightly acid air-dry soil was secured and mixed thoroughly with not very finely ground iron pyrites. This mixture was divided into two parts of which one part was inoculated with a soil-compost extract known to contain sulfur-oxidizing organisms. An equal amount of a slightly acid air-dry soil was mixed with flowers of sulfur, divided, and one part treated in a similar way as is described above. The mixture contained 50 parts of soil and 100 parts of sulfur, while the amounts of pyrites used were calculated to contain the same quantities of sulfur. The iron pyrites consisted of 45.6 per cent of sulfur and 47.8 per cent of iron. The water-holding capacity of the mixtures was dedetermined according to the Hilgard method and the moisture content kept at the supposed optimum of 60 per cent of the water-holding capacity by adding twice a week the amounts of water lost. The triplicate mixtures were placed in Erlenmeyer flasks closed with cotton plugs, stirred once every two weeks and kept at 28°C. during the incubation period of 14 weeks. Determinations of hydrogen-ion concentration, relative acidity and water-soluble sulfates were made at intervals of two weeks. The relative acidity and hydrogen-ion concentrations were determined in the manner described in an earlier paper (10). The water soluble sulfates were determined in the following way:

> The composts were thoroughly mixed and 4 grams transferred to a 500-cc. flask with about 200 cc. distilled water and 5 cc. HCl (22°B). The flasks were vigorously shaken and left standing for 12 hours; filtered into flasks of 250-cc. capacity, an aliquot drawn off, and precipitated with barium chloride. The precipitate was filtered off, washed and calcined, dried and weighed as barium sulfate.

The data secured are given in table 1.

TABLE 1

Comparative effect of inoculation on the production of water-soluble sulfates in mixtures of soil and iron pyrites and of soil and sulfur

PERIOD OF INCUBATION	SOIL AND IRON PYRITES COMPOST							SOIL AND SULFUR COMPOST						
	Not inoculated			Inoculated				Not inoculated			Inoculated			
	Reaction		Soluble sulfate	Reaction		Soluble sulfate	Increase over check	Reaction		Soluble sulfate	Reaction		Soluble sulfate	Increase over check
weeks	*pH*	*cc.**	*per cent*†	*pH*	*cc.*	*per cent*	*per cent*	*pH*	*cc.*	*per cent*	*pH*	*cc.*	*per cent*	*per cent*
0	6.1	8.9	0.63	6.1	8.9	0.63	....	4.6	23.4	0.13	4.6	23.4	0.13	....
2	6.0	9.2	0.88	5.7	14.2	1.96	1.08	4.5	24.8	0.12	3.9	36.1	0.88	0.66
4	6.0	9.4	0.97	4.4	16.2	2.47	1.50	4.7	26.2	0.16	2.6	172.1	2.32	1.16
8	5.9	9.7	1.12	3.6	20.5	3.27	2.15	4.4	25.8	0.15	2.1	279.5	2.82	2.67
12	5.8	10.9	1.26	3.9	28.3	4.12	2.86	4.4	26.0	0.19	2.0	322.2	3.22	3.03
14	5.7	12.2	1.38	4.2	47.5	4.65	3.27	4.4	27.2	0.21	2.1	305.9	3.66	3.45

* Acidity is expressed in cc. of 0.10 *N* NaOH required to neutralize 100 gm. of mixture.
Total sulfur content was taken as 100%.

It is evident that, under these conditions, oxidation of iron pyrites took place in the uninoculated mixtures, as is indicated in the change of hydrogen-ion concentration, the increase in relative acidity and the per cent of water-soluble sulfates formed after 14 weeks. The increase was gradual, as was the case in the inoculated mixture. However, the change in pH values in the inoculated mixtures of soil and iron pyrites was considerably greater, and the relative acidity increased more rapidly. The water-soluble sulfates formed had increased at the end of 14 weeks to more than 3 times the sulfates formed in the uninoculated mixture. The total water-soluble sulfates of the uninoculated mixtures after 14 weeks was but 1.38 per cent of the sulfur present, while the total water-soluble sulfates of the inoculated mixtures after the same time of incubation had increased to 4.65 per cent of the sulfur present. The mixtures were less frequently aerated in order to see whether or not the inoculated mixture would produce more sulfates under the circumstances than the uninoculated pyrite-soil mixture.

The pH values in the inoculated mixtures went down to 3.6 after 8 weeks and from then on went up again, undoubtedly because the action of the acid upon the pyrites constitutes a buffer action.

In the uninoculated soil and sulfur mixtures the pH value went down gradually also, accompanied by a slight increase in relative acidity and water-soluble sulfates. It should be stated here that it was difficult to keep the uninoculated mixtures free from contamination. It is possible therefore that after some time the uninoculated mixtures contained sulfur-oxidizing organisms. In view of this possibility the cultures were discarded after 14 weeks. The inoculated sulfur-soil mixtures increased rapidly in acidity as shown by the higher hydrogen-ion concentration and the titrated acidity. The sulfur present was oxidized at nearly the same rate as in the case of the inoculated mixtures of iron pyrites and soil, the former being but 0.18 per cent more at the end of 14 weeks. If the mixtures had been more frequently aerated this difference would have been greater as indicated by other experiments. All mixtures remained closely packed and were not stirred except for taking samples.

As could be expected, the hydrogen-ion concentration was considerably higher in the sulfur mixtures on account of the lack of material, except for the soil constitutents, with which the sulfuric acid might react. No attempt was made, however, to make a careful study of the possible increase in soluble potassium, iron, phosphorus, etc., present in the soil.

A second series conducted in tumblers covered with glass plates gave essentially the same results, but contamination was more evident after 10 weeks.

From the results obtained, the conclusion was drawn that sulfur-oxidizing organisms are active in the transformation of iron sulfides into iron sulfates when the pyrites are mixed with soil and kept at a supposed optimum moisture-content.

Series 2. Pyrites composted with a mixture of soil, sulfur and rock phosphate

As has been pointed out it was interesting to find out what the influence of iron pyrites would be on the availability of rock phosphate when composted with a mixture of soil and sulfur. A series of experiments was conducted in which 100 parts of soil were mixed with 400 parts of rock phosphate and different amounts of sulfur and pyrites. For this purpose a slightly alkaline calcareous soil was used and the sulfur replaced by pyrites so as to have approximately the same amounts of sulfur in all experiments. The exact quantities of pyrites used together with the data secured in 12 weeks are given in table 2. The relative acidity and hydrogen-ion concentration was determined at intervals and the available P_2O_5 determined after 9 and after 12 weeks. One series of cultures was kept at room temperature and another series with the same additions incubated in darkness at 30°C. The pH values for the cultures incubated at 30°C. had after 5 weeks reached the point at which the phosphoric acid becomes available. The relative acidity increased rapidly until the end of 7 weeks but from then on increased less rapidly. This could be expected on account of the neutralization of the acid by the tricalcium phosphate present. At the end of 9 weeks and at the end of 12 weeks there was no appreciable difference in total soluble P_2O_5 and acidity in any of these cultures, and they did not appreciably differ from the cultures without pyrites used as checks. The same was true of the cultures kept at room temperature. The influence of temperature was very pronounced in these cultures. In

TABLE 2

Influence of iron pyrites composted with a mixture of soil, sulfur and rock phosphate on the availability of P_2O_5

NUMBER	MATERIALS IN ADDITION TO PHOSPHATE AND SOIL†	INITIAL REACTION		AFTER 9 WEEKS			AFTER 12 WEEKS		
				Reaction		Soluble P_2O_5	Reaction		Soluble P_2O_5
		Incubated at 30°C.							
		pH	*cc.**	*pH*	*cc.*	*per cent*‡	*pH*	*cc.*	*per cent*
1	90 sulfur, 60 pyrites	6.9	0.0	3.1	178.0	9.39	3.0	198.4	10.62
2	80 sulfur, 80 pyrites	6.9	0.0	3.1	174.0	8.93	3.1	189.8	9.52
3	70 sulfur, 100 pyrites	6.9	0.0	3.3	152.0	8.28	3.1	175.3	9.94
4	60 sulfur, 120 pyrites	6.9	0.0	3.0	171.0	9.20	3.0	189.4	10.62
5	120 sulfur	6.9	0.0	3.2	161.2	8.88	3.0	180.1	9.98
		Incubated at room temperature							
6	90 sulfur, 60 pyrites	6.9	0.0	5.6	7.6	0.00	4.6	48.7	0.76
7	80 sulfur, 80 pyrites	6.9	0.0	5.4	16.5	0.00	4.0	53.2	1.96
8	70 sulfur, 100 pyrites	6.9	0.0	4.9	14.4	0.00	3.7	48.6	2.02
9	60 sulfur, 120 pyrites	6.9	0.0	5.2	10.1	0.00	3.8	21.5	1.88
10	120 sulfur	6.9	0.0	5.0	14.2	0.00	3.7	49.4	1.98

* Acidity is expressed in cc. of 0.10 *N* NaOH required to neutralize 100 gm. of mixture.

† All mixtures contained 100 parts of soil and 400 parts of rock phosphate.

‡ Total P_2O_5 content was taken as 100 per cent.

the cultures incubated at toom temperature but little P_2O_5 had been made soluble after a period of 12 weeks. However, the accumulation of acidity in the mixtures with pyrites was as gradual as in the mixtures without pyrites, being approximately the same in most of the cultures. It seemed therefore that the iron pyrites did not interfere with the formation of sulfates, nor with the availability of phosphoric acid.

Series 3. Substitution of ammonium sulfate for soil

A series of experiments similar to those reported in a previous paper (10) table 5 was conducted with a mixture of 10 parts of soil, 400 parts of rock phosphate, 50 parts of sulfur and 100 parts of iron pyrites, and 0.2 per cent ammonium sulfate. This latter salt was used to replace the nitrogen supplied by the 90 additional parts of soil used in the earlier experiments. The mixtures were incubated at 30°C. and at room temperature. Two tumblers in duplicate received 0.2 per cent of ammonium sulfate and to two of them was added also at the beginning of the incubation period 10 cc. of sulfuric acid per 100 grams of mixture. The pyrites had a lower pH value than the soil and rock phosphate, which resulted in making the mixtures slightly acid. Relative acidity and hydrogen-ion concentration were determined at intervals of two weeks and available phosphoric acid at the end of 10 weeks. The results are reported in table 3.

TABLE 3

Effect of partial replacement of soil by ammonium sulfate in compost of soil, rock phosphate, sulfur and iron pyrites

NUMBER	TREATMENT†	INITIAL REACTION		REACTION AFTER 2 WEEKS		REACTION AFTER 4 WEEKS		REACTION AFTER 6 WEEKS		REACTION AFTER 10 WEEKS		SOLUBLE P_2O_5	REACTION AFTER 12 WEEKS	
		*cc.**	*pH*	*cc.*	*pH*	*cc.*	*pH*	*cc.*	*pH*	*cc.*	*pH*	*per cent‡*	*cc.*	*pH*
	Incubated at 30°C.													
1	None	0.2	6.9	2.8	6.4	41.2	4.2	74.5	3.8	92.1	3.8	5.12	101.2	4.0
2	0.2 per cent $(NH)_2SO_4$	0.6	6.7	43.5	4.1	76.7	3.8	127.3	3.6	132.2	3.3	9.54	153.9	3.9
	Incubated at room temperature													
3	None	0.2	6.9	1.9	6.6	20.6	5.2	37.0	4.1	87.0	3.8	4.86	99.7	4.1
4	0.2 per cent $(NH)_2$ SO_4	0.6	6.7	8.9	5.5	44.7	4.1	45.6	4.0	115.5	3.4	6.26	159.6	3.7
5	0.2 per cent $(NH_4)_2$ SO_4, 10 cc. H_2SO_4 per 100 gm. mixture	1.8	6.6	2.8	6.4	43.7	4.5	44.6	4.0	116.5	3.4	6.58	140.6	3.8

* Acidity is expressed in cc. of 0.10 *N* NaOH required to neutralize 100 gm. of mixture.

† All composts contained 400 parts rock phosphate, 50 parts sulfur, 100 parts iron pyrites and 10 parts soil or soil and ammonium sulfate.

‡ Total P_2O_5 content was taken as 100 per cent.

The influence of ammonium sulfate was noticeable from the beginning in the cultures kept at room temperature as well as in the cultures incubated at 30°C. The composts incubated at 30°C. which received 0.2 per cent of ammonium sulfate had accumulated an acidity at the end of 10 weeks equivalent to 132.2 cc. of 0.5 *N* NaOH as against an acidity equivalent to 92.1 cc. 0.5 *N* NaOH accumulated by the cultures with no ammonium sulfate.

The available phosphoric acid for these mixtures at the end of the same period was 9.54 per cent and 5.2 per cent respectively.

Here again the strength of the acid formed, as indicated by the lower pH values after 10 weeks of incubation, was greater than after 12 weeks, although the total acidity had increased considerably in the period between 10 and 12 weeks. It was at first thought that this was a mistake, but the figures given represent an average of 6 determinations. The measurement of hydrogen-ion concentration is a measuring of free acid at the time of the determination, and this changes continuously. Besides, the buffer action in the mixture may cause different readings at different times. Hydrogen-ion concentration measurements may indicate at which point of acidity accumulation phosphoric acid becomes available and in this way may afford a means to follow the progress of bacterial activities; but they have, naturally, no value as measurements of the quantities of acid and acid salts produced.

Series 4. Aeration

The aeration experiments reported in an earlier paper (10) were repeated with a compost consisting of soil, rock phosphate, sulfur, pyrites and ammonium sulfate as were used in series III. In addition, some of the mixtures which were continuously aerated received 10 cc. of sulfurous acid per 100 grams of the mixture at the beginning of the experiment, and others received similar quantities of sulfurous acid in the stream of moist air used for aeration. The apparatus used in the earlier work (10) was used to provide the mixtures with moist air. The mixtures which were placed in tumblers and to which 10 cc. sulfurous acid were added per 100 grams of mixture received these quantities at the beginning or by stirring into the mixtures during the first 8 weeks. All composts were placed at room temperature and kept in darkness.

The results obtained are reported in table 4. It is evident from this table that aeration of the mixtures by means of a stream of air had very little or no beneficial influence upon the availability of the phosphoric acid. Contrary to the experiments reported before (10) these aerated mixtures accumulated considerable acidity, provided ammonium sulfate was added. These particular mixtures had made available an average of 10.45 per cent phosphoric acid at the end of 10 weeks. This could not be attributed to the abundance of air acting on the pyrites for the cultures without ammonium sulfate received exactly the same amounts of air. The effect is ascribed to the pyrites acting as catalyser and thereby favoring the action of the sulfur-oxidizing organisms. As evidence for this assumption it may be stated that

TABLE 4

Influence of continuous aeration on the accumulation of acidity and available phosphoric acid

NUMBER	TREATMENT†	INITIAL REACTION		REACTION AFTER 2 WEEKS		REACTION AFTER 4 WEEKS		REACTION AFTER 6 WEEKS		REACTION AFTER 8 WEEKS		REACTION AFTER 10 WEEKS		REACTION AFTER 12 WEEKS		SOLUBLE P_2O_5
		*cc.**	*pH*	*cc.*	*pH*	*cc.*	*pH*	*cc.*	*pH*	*cc.*	*pH*	*cc.*	*pH*	*cc.*	*pH*	*per cent*‡
1	Stream of moist air	0.6	6.7	4.6	5.9	12.4	5.6	16.8	5.1	22.4	5.0	36.2	4.8	59.4	4.2	0.62
2	0.2 per cent $(NH_4)_2SO_4$; stream of moist air	0.6	6.7	47.1	4.4	44.1	4.7	82.1	3.6	130.1	3.5	141.5	3.2	163.6	3.4	10.45
3	0.2 per cent $(NH_4)_2SO_4$; 10 cc. sulfurous acid added at beginning, stream of moist air	0.2	6.9	2.5	5.9	39.7	4.8	23.0	5.0	120.0	3.6	130.1	3.2	145.5	3.5	8.26
4	0.2 per cent $(NH_4)_2SO_4$; 10 cc. sulfurous acid in container through which air was running	0.2	6.9	70.1	4.3	66.7	5.0	65.2	3.7	59.8	3.9	57.4	3.8	62.5	4.0	0.78
5	None	0.6	6.7	0.6	6.6	1.0	6.4	2.8	5.5	16.1	5.0	34.0	3.9	51.1	4.0	0.32
6	0.2 per cent $(NH_4)_2SO_4$	0.6	6.7	45.8	4.5	44.9	4.7	52.4	3.9	94.6	3.6	120.3	3.3	153.3	3.4	9.32
7	0.2 per cent $(NH_4)_2SO_4$; 10 cc. sulfurous acid added at beginning	0.2	6.9	4.0	5.7	42.8	4.8	57.1	3.9	49.7	3.8	53.2	3.9	130.4	3.5	7.81
8	0.2 per cent $(NH_4)_2SO_4$; 10 cc. sulfurous acid added by stirring	0.2	6.9	3.4	6.0	36.1	4.9	47.9	3.9	44.4	3.9	47.9	4.0	123.9	3.8	5.76

* Acidity is expressed in cc. of 0.10 *N* NaOH required to neutralize 100 gm. of mixture.

† All composts contained 400 parts rock phosphate, 50 parts sulfur, 100 parts iron pyrites and 10 parts soil or soil and ammonium sulfate.

‡ Total P_2O_5 content was taken as 100 per cent.

qualitative analyses of the mixtures showed that great quantities of iron sulfates were present. This was undoubtedly due to the activities of the microörganisms, for but comparatively small amounts of iron sulfates were present in mixtures numbers 1 and 5 which received no ammonium sulfates as a source of nitrogen and which in consequence produced but little acidity.

The addition of sulfurous acid failed to stimulate these mixtures, both in the composts continuously aerated and in the mixtures kept in tumblers. Sulfurous acid stirred into the mixtures or added in the stream of moist air proved to be depressing rather than stimulating. The action of sulfurous acid therefore, seems in the first place of a sterilizing nature. Especially where small amounts of soil are used this action is more pronounced.

Series 5. Vegetation experiments

Since iron is one of the absolutely essential elements to normal growth and development of all agricultural plants, but since the quantities of iron in the soil are usually so large and supposed to be available in sufficient amounts to perform the necessary functions, it is not often applied as a fertilizer.

Strenuous efforts have been made by the manufacturers of certain products to introduce iron sulfate, which is a common by-product of a number of manufacturing processes, as a fertilizer. Pyrite cinders have been used in many places with success, especially in the Aisne region in France. Their value has been attributed both to the iron sulfate and to small amounts of nitrogen which these cinders contain.

Vivien (9) mixed 1 per cent of roasted pyrites with manure and found that considerable amounts of nitrates were formed, whereas in manure treated with acid phosphate, iron sulfate, calcium sulfate, and lime, only traces of nitrates were found.

Vermorel and Dantony (8) employed iron pyrites at rates of 100 and 200 kgm. per hectare as a top-dressing for wheat and beans. In one series they employed pure sand with additions of 50 kgm. sodium nitrate per hectare and in another series 100 kgm. dried blood per hectare. The pyrites was applied as a top dressing and mixed with the sand.

The pyrites increased the yields of wheat 40 per cent and for beans 50 per cent. These investigators conclude that the sulfur of the pyrites acted as a stimulant. They found however, that pyrites alone was more effective than sulfur alone on wheat and less effective on beans. Best results were obtained with a mixture of sulfur and pyrites.

Since these investigators had better results with a mixture of pyrites and sulfur the question can be raised whether this was due to the iron in a form more available to the plants. Van Alstine (6) concludes from his solution culture work that with a limited supply of iron in the form of ferric hydroxyphosphate "a hydrogen-ion concentration of 4.5 is as low as can be expected to dissolve the amounts of iron necessary for proper growth of buckwheat, soy beans and wheat. With lower hydrogen-ion concentrations so little iron is dissolved that these plants are unable to get the amounts they need and begin to show chlorosis as soon as the supply in the seed is used up." The form in which iron is supplied is very important as is shown by Jones and Shive (4) in their nutrient solution work. In the nutrient solution employed, iron in the form of ferric sulfate was very slowly and difficultly available to wheat plants even when supplied in relatively large quantities, but ferrous sulfate appeared to be readily available to these plants. It might be that the good results of Vermorel and Dantony with pyrites were caused by the iron changing from the sulfide into the sulfate form and thereby becoming more readily available, for they used paraffined pots with certain quantities of pure sand which was carefully freed from organic material. Through the washing of the sand they had naturally removed the iron present. That sufficient iron in the soil is not always available to plant growth is shown by the results obtained bv Chanzit (2) with vines which showed chlorosis. He found that the presence of excessive amounts of $CaCO_3$ in the soil caused chlorosis to the vines, which he could overcome by applying 250–300 gm. of ferrous sulfate to each vine during the winter.

An experiment was carried on in the greenhouse in earthenware pots with washed quartz sand. Shive's nutrient solution (R_5C_2) was used as basis. The following treatments were used (the numbers refer to the pot cultures):

1. None.
2. Pyrites.
4. Nutrient solution (R_5C_2).
5. Nutrient solution and pyrites.
6. Nutrient solution, but phosphorus replaced by ground rock phosphate.
7. Nutrient solution, but phosphorus replaced by ground rock phosphate, and pyrites.
9. Nutrient solution, but phosphorus replaced by ground rock phosphate, inoculated sulfur, and pyrites.

The pyrites were added at a rate of 200 pounds per acre. The amount of rock phosphate employed was 2 tons per acre, calculated to be approximately the same as in the nutrient solution. Sulfur was added at a rate of 100 pounds per acre.

Soy beans were grown for 6 weeks and the yields obtained are recorded in table 5, together with other data obtained.

Although the yields of tops were but slightly higher for the cultures receiving pyrites in addition to the nutrient solution, they were markedly earlier in maturing. After 4 weeks all plants to which pyrites were added were blooming, while but one plant of the other cultures started to bloom at that time. This earlier maturing is also indicated by the number of pods produced after 6 weeks. The plants receiving inoculated sulfur and pyrites were dead after 18 days. The acidity produced was apparently too high for these plants.

Although no conclusions could be drawn from these experiments, since they were of a too limited scope, they seem to point to the possibility that at least a part of the increase in yields obtained by Vermorel and Dantony is caused by a change of the iron to more soluble forms, since no iron was applied at the beginning of the experiments, conducted by these investigators.

TABLE 5

Yields of soy bean tops grown in sand cultures for 6 weeks with Shive's nutrient solution as basis, and with additions of pyrites

POT NUMBER	TREATMENT	DRY WEIGHT OF TOPS	AVERAGE HEIGHT	NUMBER OF PODS	INITIAL REACTION	FINAL REACTION
	per acre	*gm.*	*cm.*		*pH*	*pH*
1	None	0.850*	8	Bloom	5.8	6.7
2	Pyrites, 200 lbs.	0.990	7.5	Bloom	5.4	6.5
3	R_5C_2	3.342	14	3	5.4	6.5
4	R_5C_2, pyrites, 200 lbs.	3.610	15	9	5.2	6.6
5	R_5C_2, rock phosphate, 2 tons; pyrites, 200 lbs.	2.022	10.5	2	4.9	6.4
6	R_5C_2, rock phosphate 2 tons; sulfur, 100 lbs.; pyrites, 200 lbs.	0.704	dead		5.0	3.3

* All measurements are an average of three cultures.

CONCLUSIONS

1. From these studies it seems evident that iron pyrites can be attacked by microörganisms and changed into the sulfate form. No attempt however, was made to study the intermediate steps in the changes occurring. If small quantities of sulfur are added these changes are much more rapid.

2. Pyrites composted together with sulfur and rock phosphate do not interfere with the gradual increase in acidity formation nor with the increase in availability of phosphoric acid.

3. The replacement of soil with ammonium sulfate in composts in which quantities of sulfur are replaced by iron sulfide produced a marked increase in available phosphoric acid. The effect, when sufficient nitrogen is present for the needs of the microörganisms, is ascribed partly to the pyrites acting as a catalyser and as such favoring the action of sulfur-oxidizing organisms, and partly to the changes from the sulfide into the sulfate form.

4. Aeration of sulfur-pyrites-rock phosphate compost mixtures by means of a continuous stream of air has little or no beneficial effect upon the production of acidity and consequent availability of soluble P_2O_5, unless ammonium sulfate is added.

5. The action of sulfurous acid in such mixtures seem to be mainly of a sterilizing nature.

REFERENCES

(1) ALLEN, E. T., AND JOHNSTON, J. 1910 The exact determination of sulfur in pyrite and marcasite. *In* Jour. Indus. and Engin. Chem., v. 2, p. 196–199.

(2) CHANZIT, J. 1913 Chlorose de vignes et leur traitément dans l'hiver. *In* Rev. Vit., v. 38, p. 761–763.

(3) LÖHNIS, F. 1910 Handbuch der Landwirtschaftliche Bakteriologie, p. 705–708. Bornträger, Berlin.

(4) JONES, L. H., AND SHIVE, J. W. 1921 The influence of iron in the form of ferric phosphate and ferrous phosphate upon the growth of wheat in a nutrient solution. *In* Soil Sci., v. 11, p. 93–99.

(5) Kappen, H., and Quensell, E. 1915 Über die Umwandlungen von Schwefel und Schwefelverbindungen im Ackerboden. *In* Landw. Vers. Stat., v. 86, p. 1–34.
(6) Van Alstine, E. 1920 The interrelation between plant growth and the hydrogen-ion concentration of nutrient solutions. Thesis, Rutgers College, New Brunswick, N. J.
(7) Van Bemmelen, J. 1886 Bijdragen tot de kennis van den alluvialen bodem in Nederland. Holkema and Warendorf, Amsterdam.
(8) Vermorel, V., and Dantony, E. 1913 Contribution à l'étude du soufre et des pyrites de fer employés comme engrais. *In* Engrais, v. 28, p. 1304–1306.
(9) Vivien, A. 1905 Fumier. *In* Monit. Sci., ser. 4, v. 19, p. 773–779.
(10) Rudolfs, W. 1922 Composting of rock phosphate with sulfur in slightly alkaline, calcareous soils. *In* Soil Sci., v. 14, p. 37–59.

PLATE 1

PLANTS GROWN IN SAND CULTURES WITH SHIVE'S NUTRIENT SOLUTION AS BASIS AND WITH ADDITIONS OF PYRITES

Treatments were as follows: (1) none; (2) pyrites; (4) R_5C_2; (5) R_5C_2 and pyrites; (7) R_5C_2 and rock phosphate; (9) R_5C_2, rock phosphate-sulfur mixture and pyrites.

5
7
9

A MICROSCOPIC METHOD FOR DEMONSTRATING FUNGI AND ACTINOMYCETES IN SOIL[1]

H. J. CONN

New York (Geneva) Agricultural Experiment Station

Received for publication October 24, 1921

A few years ago a microscopic method for examining soil was proposed by the writer (2). Dried and stained microscopic mounts were used. Surprise was expressed at the time that practically no mold hyphae were observed in ordinary soils. It was remarked that there was no question but that fungi can live in the soil, and that the failure to find fungi in microscopic preparations did not disprove their activity in agricultural soils in general. In spite of such comments, however, the paper has been interpreted in some quarters as denying the activity of fungi in soils.

As the writer has always believed in their activity in soil under favorable conditions, it seemed well to continue the work by improving the methods of determining the presence of active fungi in soil, and to notice under what conditions they are most abundant. The criticism has been raised against the above mentioned microscopic technic that it might fail to show fungus filaments even if present—they might be torn off the slide in washing, they might be so affected by drying as to fail to stain, or the fungi might even occur in some vegetative form not easily recognized. That this criticism is not entirely justified may be shown by sterilizing a small quantity of soil in a test tube, inoculating it with mold spores of almost any species, and then after incubation examining under the microscope by the method in question. The presence of fungous hyphae is easy to demonstrate in any such inoculated soil, where they are known to be growing actively.

In spite of such findings, however, there seemed a possibility that there might be some truth in the claim that when but small quantities of filaments are present they may be washed off the slide and fail to show in the finished preparation. To meet this criticism, therefore, an improved technic has been developed, using wet mounts instead of dry. The technic is as follows:

Place a small crumb of soil (10 mgm. or less) on a microscopic slide. Mix it with two or three drops of water. Then dip a small glass rod into a methylen blue solution (either saturated aqueous or the Loeffler solution), and introduce the rod into the drop of soil infusion on the slide. Mix well together and cover with a cover slip, removing any sand grains that would prevent the cover slip from resting level. The strength of the stain on the slide should be such that the mount appears distinctly blue to the naked eye, but the field is only slightly tinted when viewed through the microscope. If too much stain has been added, it may be washed out without removing the cover glass by placing a drop of water on one side and touching the other side with a piece of filter paper. Examine with a dry lens and a highpower eye piece. The combination of lenses that has proved best for general purposes is a 16 mm. objective with a 15x compensating eye-piece.

---

[1] This and the following paper appear together by mutual request of the authors.—Ed.

By using this technic, fungus filaments, or at least fragments of them, have been observed in nearly all the soils that have been examined—a finding more nearly in accord with what was to be expected than was the observation made with the earlier technic. In the greater number of cases, however, the filaments were far from abundant. Sometimes in the whole preparation (comprising at least 5 mgm. of soil) only four or five fragments of mycelium were observed. The cases where they were found in sufficient abundance to suggest that they might have been playing a prominent part in the soil activities were those which would naturally be predicted; namely, where large amounts of undecomposed organic matter were present. By this same technic it was found that actinomyces filaments are abundant in some soils and entirely lacking in others, although plate counts always show large numbers of these organisms.

These observations emphasize even more than previous work the fact that plate counts of spore-forming organisms give no real idea as to their activity in any substance under investigation—be it soil or any other habitat of microorganisms. By using this microscopic technic it is possible, for example, to demonstrate an increase in the number of actinomycetes in soil in which young grass is growing. The plate count, to be sure, as pointed out some time ago (1), shows larger numbers of actinomycetes in sod than in cultivated soil; but this difference was never noticed until the sod was two or three years old. The microscope indicates that the increase in their activity takes place early in the life of the grass, but that their spores do not greatly increase in numbers for some time.

The promising feature in connection with the new technic is that it offers a rapid and apparently accurate method of determining the presence of vegetative of fungi and actinomycetes. The plate count gives misleading conclusions, as it is really a count of spores; the dried microscopic preparations show the filaments only when they are exceptionally abundant; the wet mount method is the only means yet at our disposal for getting direct evidence as to the extent of their vegetative activity. By using it, there should be no trouble in learning which soil conditions specially favor the development of these filamentous organisms.

SUMMARY

It is generally agreed that spores of fungi are universally present in soil and that they are capable of growing there under proper conditions. The presence of these spores in itself indicates activity at some recent date, as they are found too deep in the soil to be the result of air contamination without growth.

Naked-eye evidence shows that certain fungi, such as mushrooms, puffballs, etc., can thrive in soil, especially if it is well supplied with woody material or cellulose. This does not, however, necessarily indicate large activity of fungi in ordinary cultivated field soil to which little organic matter has been added, since the microscope shows mycelium to be present in but small quantities in such soil.

It must be acknowledged that fungi are always a potential factor, and possibly an important factor in soil fertility even though in ordinary cultivated soil their filaments may be few and their activities overshadowed by those of bacteria. In order to learn when and where they become active, instead of merely potential, factors in the soil activities, a simple method is needed for demonstrating the abundance of their vegetative forms, so as to correlate their abundance with the chemical transformations known to be occurring in soil. It is felt that the present technic supplies this much needed method.

REFERENCES

(1) Conn, H. J. 1916 A possible function of actinomycetes in soil. N. Y. Geneva Agr. Exp. Sta. Tech. Bul. 52; *also in* Jour. Bact., v. 1, p. 197–207.

(2) Conn, H. J. 1918 The microscopic study of bacteria and fungi in soil. N., Y. Agr. Exp. Sta. Tech. Bul. 64.

THE GROWTH OF FUNGI IN THE SOIL[1,2]

SELMAN A. WAKSMAN

New Jersey Agricultural Experiment Stations

Received for publication October 24, 1921

A few years ago, the writer published a note (6) relative to the fact that fungi exist in the soil not only in the form of spores, but also as mycelium, which indicates that these organisms are active in the soil and take a part in the soil fertility processes. A method has been suggested for the demonstration of this fact, and, by the use of this method, which is quite simple, a number of fungi have been found to exist in the soil in the form of vegetative mycelium. The majority of the organisms isolated by this method were found to belong to the *Mucorales* and were included in the genera *Mucor*, *Rhizopus* and *Zygorhynchus*. Such large groups of soil organisms as the *Penicillia*, *Aspergilli* and *Cladosporia* were not obtained at all or only in very few instances by this method. This would point to one of two considerations: either the *Mucorales* develop more rapidly out of the soil and thus crowd out the other organisms or, that the *Mucorales* and a few other fungi, such as *Trichodermae* are present in the soil in the form of vegetative mycelium, while the *Penicillia*, *Aspergilli* and *Cladosporia* are present there only in the form of spores.

When the common plate method is used for the study of soil fungi, the opposite may be found to hold true: the *Mucorales* may not develop to such an extent as the *Penicillia*, *Aspergilli* and *Cladosporia*, while such fungi as the green *Trichodermae* are obtained by both methods. The following hypothesis then suggested itself: the *Mucorales* and *Trichodermae* are always present in the soil in the form of spores and vegetative mycelium, the various representatives of these groups preferring one or another soil type or different environmental conditions; the *Penicillia*, *Aspergilli* and *Cladosporia*, which are common air inhabitants and are found abundantly above the surface of the soil, in the dust, are always present, one group or another, in the soil in the form of spores, which may germinate and produce a vegetative mycelium, when soil conditions become favorable, as in the case of addition of organic matter, proper moisture content, etc.

Since the first note was published, several reports were made by the writer (7, 8, 9), in which a study was made of the fungous flora of the soil and it was

---

[1] Paper No. 96 of the Journal Series, New Jersey Agricultural Experiment Stations, Department of Soil Chemistry and Bacteriology. This paper will appear in Rutgers College Studies, Vol. 1.

[2] This and the preceding paper appear together by mutual request of the authors.—ED.

established without any doubt, that fungi (or molds) are permanent inhabitants of the soil and take part in important soil processes. Forms isolated in one locality may also be found in soils thousands of miles away, under entirely different environmental and soil conditions. However, the common plate method used with such convenience for the study of soil bacteria is entirely inadequate for the study of fungi, since not only the numbers but even the species of fungi developing on the common agar or gelatin plate do not give a true indication of the relative abundance and possible importance of fungi in the soil. This is due to the fact that the mycelium in the soil, however abundant, may be entirely absent, when the soil is diluted 10,000 or more times, for plating out of bacteria, while the fungi developing on the plate indicate only the spores of fungi present in the soil. These are so unevenly distributed that great variability is found not only throughout one soil sample but even on plates made from the same dilution, so that one plate may contain two or three colonies and a duplicate plate eight or ten fungus colonies.

In addition to the method suggested by the writer for the demonstration of fungus mycelium in the soil, a direct microscopic method has been suggested by Conn (2). The later investigator (1, 2), however, was unable to demonstrate any mold hyphae in the soil, which led him at first to conclude that molds are of relative insignificance in the soil. When one considers the fact that, for microscopic examinations, only a very small fragment of soil can be used and this is further diluted with water, one is not surprised that hyphae are not obtained by the microscopic method. Under these conditions any mycelium which would be present in a larger granule of soil, would be in most cases eliminated, since the mycelium is not so readily broken up into fragments as a bacterial chain. Then, mold hyphae assume in the soil a somewhat different appearance than in pure culture and might easily be mistaken, in a stained preparation, for organic matter.

The current idea about soil fungi was that these organisms are abundant only in soils of acid character and well supplied with organic matter. This is quite right, but it has also been pointed out in previous publications of the writer and others (3) that fungi are present in neutral and alkaline soils as well as in sandy soils containing very little organic matter. The following results will show that, 1, fungi are present in a mycelial stage in the soil and, 2, fungi are present abundantly not only in acid soils and those rich in organic matter, but even in nearly pure silicious sands not only in the form of spores but as abundant mycelium and also in neutral soils.

The author's method used for demonstrating fungi is, briefly, as follows: a clump of soil, the size of a large pea, taken out carefully from the soil sample with as little contamination from the air as possible, is placed in the center of a sterile plate, into which 10–12 cc. of a sterile nutrient agar, favorable for the development of fungi, has been placed; the soil is slightly pressed into the agar, so as to be surrounded by the nutrient medium. The plates are incubated at 25–30°C. At the end of 24–26 hours' incubation, the plates are examined. Mold hyphae are then found to radiate out of the clump of soil into the surrounding medium. This is based upon the fact that the fungi present in the soil in the form of mycelium will grow at once into the medium, before the spores can germinate and develop hyphae. When a small piece of agar containing the growing mycelium, preferably a tip of a growing hyphae as far from the clump of soil as possible, is transferred upon a sterile slant of agar practically a pure culture of the particular fungus may be obtained.

One of the most common soil fungi found in the soil by the above method is a species of *Zygorhynchus*, closely related (5) to *Zygorhynchus vuilleminii* (Namys). A somewhat different species has been commonly found in northern soils obtained from Alberta (Canada) and Alaska. This fungus has been found by the direct method in practically all the soils examined, independent of the fact whether the soil is rich or poor in organic matter, acid, neutral or alkaline in reaction. Of special interest is the fact that this organism is found abundantly in subsoils, particularly sandy subsoils. Invariably a pure culture of this organism has been obtained by placing a clump of sandy subsoil, even as far as thirty inches deep, upon the plate.

Recently a sample of soil was received from Lakewood, N. J. It was obtained from an excavation and was stated to be present there in large quantities; its character was so unusual that an analysis was asked for. The soil belongs to the Lakewood series of sandy soils described in the soil Survey of the Freehold area (4), and is nearly a pure white sand, so typical of the New Jersey pine barrens. This particular sample, which was obtained in a sand excavation and was stated to represent a "considerable quantity" was found to be clumped together by a sort of a fine cottony mass penetrating throughout the soil. The soil had a reaction equivalent to pH 6.2 and contained, by the Kjeldahl method, 0.0123 per cent nitrogen.

This soil was examined for its content of microörganisms. The common plate method gave, in a 10,000 dilution, two or three bacterial colonies and two or three actinomycetes, per plate. A 1000 dilution gave fifteen to thirty bacterial colonics, six to ten actinomyces colonies, and one or two colonies of a green *Penicillium*. The direct method revealed the puzzle. A pure culture of the common soil *Zygorhynchus* was readily obtained and this was found to be the organism, whose mycelium penetrated the sand to such an extent that it held it in a compact mass. This organism which is found abundantly in the poor sandy subsoils of the Sassafrass series of New Jersey was found to grow to such an extent in this barren sand as to hold the sand together compactly. What rôle this organism plays in the soil is not yet known. It either decomposes the traces of organic matter present in the soil, making the nitrogen and minerals available for the scrub pines, or, as in the case of the subsoils, it merely thrives in this medium, practically free from other competing microorganisms, on the minerals washed down from the surface soil by the drainage waters. This fact, of course, would not point to any great importance of fungi in the soil, but tends to indicate that they may become readily active under favorable circumstances.

Another interesting instance of the activity of fungi in the soil is found in the following illustration. A farmer located a few miles from the Experiment Station observed the fact that, by mixing soil, undecomposed organic matter, such as clover, alfalfa, etc., and non-nitrogenous mineral fertilizer, then adding the proper amount of water, a good growth of molds takes place. When this mixture was turned over every two or three days, the molds decomposed the organic matter so rapidly that, in seven days, the whole mass was quite similar to leafmold, and compared favorable with prepared organic fertilizers for certain greenhouse plants. The farmer went so far as to apply for a patent on this process. When this mixture of soil, organic matter and mineral fertilizer was examined, it was found that a few species of *Mucor* (*M. plumbeus*, etc.), were chiefly responsible for the decomposition of the organic matter and for the artificial formation of the leafmold. The *Mucors* grew to a height of one to two inches above the surface of the mixture.

However, to be able to determine the actual number of fungi in the soil, as represented both by the numbers of spores and pieces of mycelium, a new procedure has been developed. This was a result of a series of studies on the variability of numbers of microörganisms in the soil as determined by the plate method (10). It was found that, when the numbers of fungi are determined on the plates prepared for the count of bacteria and actinomycetes, the variability is so great, due to the high dilution of the soil and, therefore, to the small numbers of fungi obtained, that the probable error obtained even from as many as 50 plates prepared from one soil is so large as to make the results worthless. Where low dilutions of the soil are employed, so many bacteria will develop on the plate as to actually crowd out a good many fungi and make the count entirely unreliable.

By the use of special acid media, on which no bacteria and actinomycetes will develop, and low dilutions (about 1000), the numbers of fungi can be determined quite accurately. Of the acid media two can be suggested:

1. Raisin agar, prepared by heating 60 gm. of raisins in 1000 cc. of tap water for 1 hour, then adding 25 gm. of agar, dissolving, adjusting the reaction to pH 4.0, filtering, tubing and sterilizing as usual.
2. Synthetic agar: 10 gm. dextrose, 5 gm. peptone, 1 gm. KH_2PO_4, 0.5 gm. $MgSO_4$, 1000 cc. of water. Enough 1.0 *N* acid (sulfuric or phosphoric) is added to make the reaction equivalent to pH 3.6–3.8. This will require about 5–7 cc. 1.0*N* acid per liter of medium. Twenty-five gm. of agar are then added and dissolved by boiling. The medium is then filtered through cotton, tubed and sterilzied as usual. The reaction of the medium after sterilization should be pH 4.0.

By the use of the two acid media and a low dilution of soil (2–0.5 per cent of that used for counting of bacteria), plates can be obtained containing only fungus colonies. The number of fungus colonies per plate should be between 30 and 100. Incubate 48–72 hours at 25°C. Where the majority of the colonies are of the same species, there is ground for suspicion of air blown spores, rather than soil forms.

By the use of this method, not only were the numbers of fungi present in the soil found to be lower, but the results were more definite and less variable. Even more important, a definite correlation has been found between soil treatment, soil reaction and numbers of fungi in the soil, as indicated in the following table.

TABLE 1

Influence of soil treatment upon numbers of fungi as determined by the plate method

SOIL FERTILIZATION	REACTION	NUMBERS OF FUNGI PER GRAM OF SOIL
	pH	
Minerals only	5.6	37,300
Heavily manured	5.8	73,000
Sodium nitrate	5.8	46,000
Ammonium sulfate	4.0	110,000
Minerals and lime	6.6	26,200
Ammonium sulfate and lime	6.2	39,100

Manure and acid fertilizers (ammonium sulfate) stimulate an increase in the numbers of fungi. By making the reaction less acid, lime results in a great decrease in the numbers of fungi. Further information on this method and more extensive results will be published later.

REFERENCES

(1) Conn, H. J. 1916 Relative importance of fungi and bacteria in soil. *In* Science, n. s., v. 44, p. 857–8.

(2) Conn, H. J. 1918 The microscopic study of bacteria and fungi in soil. N Y. (Geneva) Agr. Exp. Sta. Tech. Bull. 64.

(3) Dale, E. 1912–1914 On the fungi of the soil. *In* Ann. Mycol., v. 10, p 452–477; v. 12, p. 33–62.

(4) Jennings, H., Dickey, J. B. R., and Lee L. L. 1916 Soil Survey of the Freehold Area, New Jersey. U. S. Dept Agr. Bur. Soils, Field Operations, 1913.

(5) Namyslowski, B. 1910 *Zygorhynchus Vuilleminii,* une nouvelle mucorinée, isolée du sol et cultivée. *In* Ann. Mycol., v. 8, p. 152–155

(6) Waksman, S. A. 1916 Do fungi live and produce mycelium in the soil? *In* Science, n. s., v. 44, p. 320–322.

(7) Waksman, S. A. 1916 Soil fungi and their activities. *In* Soil Sci., v. 2, p. 103–155.

(8) Waksman, S. A. 1917 Is there any fungus flora of the soil? *In* Soil Sci. v. 3, p.565–589.

(9) Waksman, S. A. 1918 The importance of mold action in the soil. *In* Soil Sci., v. 6, p. 137–155.

(10) Waksman, S. A. 1922 Microbiological analysis of soil as an index of soil fertility. I. The mathematical interpretation of numbers of microörganisms in the soil *In* Soil Sci., v. 14, p. 81–101.

FIELD MOISTURE CAPACITY AND WILTING POINT OF SOILS

W. L. POWERS

Oregon Experiment Station

Received for publication November 23, 1921

The storage capacity of the soil for usable moisture under field conditions affects the amount of irrigation, necessary while the drouth, or wilting, point affects the time of irrigation and defines the lower limit of field capacity of the soil for usable moisture. The field capacity of the soil for usable moisture has come to be regarded, therefore, as one of its most important, if not the most important of physical properties. These matters have come to be given careful study in connection with Oregon Soil Investigations.

The wilting coefficient is the moisture content of the soil at which the plant wilts permanently or at which it cannot maintain its turgidity. This point has been regarded as varying but slightly with different plants, not usually more than 1½ per cent. It has commonly been regarded as varying widely with different soils. This wilting point represents the lower limit of available moisture. Briggs has shown (2) that it is approximately one and one-half times the hygroscopic coefficient. In coarse-textured soils the hygroscopic coefficient is very low and the wilting coefficient correspondingly low. In fined-texture soils these points are fairly high, the hygroscopic coefficient lying between 10 and 18 per cent, wilting occurring at 15 to 27 per cent. In some Oregon peat soils, the wilting coefficient has been found to be as high as 65 per cent. Oregon studies indicate that the wider the range in the different moisture points and the finer the texture, the greater is the difference between the wilting points of different crops grown on a given soil. With soils of coarse texture, where there is a narrow range of important moisture points, there is less difference in the wilting point of various crops. Oregon studies indicate that the difference is greater than was formerly supposed and reported (2).

WILTING POINT STUDIES UNDER FIELD CONDITIONS

The moisture history of dry-farmed and irrigated plats, including both meadow and cultivated plats, located on Willamette silty clay loam was obtained at the Oregon Station during 1910. From these data it appeared that the clover and other meadow crops did not require irrigation until the moisture content for the first two feet of soil dropped down to 14 or 15 per cent, dry weight. For potatoes the moisture content of the same strata did not reach this low point during the entire growing season on dry plats, and the plats to be irrigated showed indications of drouth, when the moisture in the first two feet was still 20 or 21 per cent. An experiment was, therefore, planned determine the drouth point of potatoes and of clover, or the point at which it was best to apply irrigation water. A series of plats in duplicate was set aside, to be cropped to potatoes, one plat to be irrigated when the moisture content of the first two feet had dropped to twenty-three per cent point; the second when the moisture content reached the twenty-per cent point, and the third when the moisture had dropped to the seventeen-per cent point. After 1911 the points at which irrigations were applied were changed to 24, 21, and 18 per cent, respectively. At the same time clover plats were laid out to be irrigated which the moisture content dropped to the 20 per cent, 17 per cent, and 14 per cent points, respectively. An experiment was conducted for the years 1911, 1912, and 1913. Data were reported in Bulletin 122 of the Oregon Experiment Station. Since it is out of print, a summary of the more important data is presented in table 1.

TABLE I.

Value of soil-moisture tests in determining the exact time to irrigate

CROP	TREATMENT	TOTAL YIELD PER ACRE	GAIN OVER DRY PLAT		GAIN PER ACRE-INCH OF WATER ADDED	WATER REQUIREMENTS
(1911)		*bu.*	*bu.*	*per cent*	*bu.*	*lbs.*
Potatoes (Recd. 3 by 3 inches)	Irrigated 23 per cent (or 9 inches)	292.5	157.4	117	17.5	1058
Potatoes (Recd. 2 by 3 inches)	Irrigated 20 per cent (or 6 inches)	308.5	173.4	128	28.9	799
Potatoes (Recd. 1 by 3 inches)	Irrigated 17 per cent	176.4	41.3	31	13.8	1326
(1913)						
Potatoes (Recd. 2 by 2 inches)	23 per cent	260.0	−40.5	−13.0	−10.1	973
Recd. 1 by 2 inches	20 per cent	342.0	41.5	18.0	10.4	655
Recd. none	17 per cent	300.5				629
(1911)						
Clover		tons	(green)		tons	
1 by 4 inches	20 per cent	17.05	6.60	63	1.85	338
1 by 4 inches	17 per cent	19.37	8.92	85	2.23	306
1 by 4 inches	14 per cent	19.62	9.17	88	2.29	303
(1913)						
2 by 5 inches	20 per cent	4.925		No dry plant, 2nd crop year		514
1 by 5 inches	17 per cent	5.175				539
1 by 5 inches	14 per cent	5.100				459

In a summer of low precipitation, 1911, and in a summer of high precipitation, 1913, irrigation of potatoes when the moisture content reached 20 per cent seemed to give the greatest increases on the Willamette silty clay loam soil, on the Corvallis experiment field, and this moisture content has come to be taken as an indicator of the exact time to irrigate this crop. During the same years it developed that clover was best irrigated when the moisture point dropped to 14 or 15 per cent for the first 2 feet; the moisture did more good when applied at these points, as the water requirement per pound of dry matter was generally lower under such conditions.

In order to check the drouth points of these crops and to eliminate any reinforcement of moisture from the subsoil secured by the deep roots of the clover plants, a series of Briggs type tanks were arranged in duplicate in 1918, and each of these crops grown in six tanks. Two tanks were irrigated when the crop was distinctly wilted, a second pair when the crop was but slightly wilted, and third pair when the crop was still in a fresh condition. Experiments were conducted in 1918 and the following year additional tanks were added for beets and alfalfa. After the crop growth was well under way, the tanks were allowed to dry down to the drouth point as indicated by the appearance of the crop at 3:00 p.m. Samples were then taken for soil-moisture determination from cores extending throughout the depth of the soil tank. The tanks were then given different amounts of irrigation to revive the plants and allowed to dry down until the average tank should again show slight wilting and the minimum tank distinct wilting, whereupon they were resampled. Representative tests are shown in table 2.

Some lack of uniformity in the data appears on account of interference of war conditions and the pressure of other work, but the average point at which the clover was found to wilt in these tanks was 16 per cent, for potatoes it was 17.5, for beets 20.3. This is the mean drought point by crops for this soil. The lower wilting point for all crops early in the season is due to low temperature (5) and relative humidity which was about 60 per cent, while later determinations were made with summer temperatures of 80° to 90°F. and a relative humidity of about 30 per cent. The water requirement was determined for potatoes and beets. The water requirement was generally lower for the crops in the average tanks. The data are sufficient to indicate strongly that the wilting point of the different crops varies more than has formerly been supposed. The indications are that it varies more widely in soils of heavy texture and wide range of important moisture points.

The wilting point as determined by laboratory methods for wheat seedlings in this Willamette silty clay loam has been found to be 15 per cent.

FIELD MOISTURE CAPACITY

The usable water capacity of nearly a score of important irrigated soils in different irrigated valleys of the state has been studied in connection with duty-of-water investigations (3) in order to secure definite information as to the best amount to apply at one time. Various factors affecting this field capacity have been measured. Cylinders 6 inches in diameter and 1 foot long.

or of approximately one-fifth cubic foot capacity were forced into the soil to a depth of 1 foot and the samples thus secured were saturated in a damp enclosure in the laboratory, drained to constant weight, and the total moisture content determined. This practice also afforded opportunity to determine the volume weight per cubic foot. Field samples taken for soil-moisture determinations before and after irrigation have given indications of the maximum and minimum range of soil moisture under field conditions, and these, used in connection with the maximum field capacity tests and other data, aid in determining the usable moisture capacity in percentage and in inches on these important irrigated soils. The data so secured are presented in table 3.

TABLE 2

Wilting points of different crops

CROP	TANK NUMBER	COMPARATIVE MOISTURE AT SAMPLING	ACTUAL MOISTURE JULY 17	CONDITION OF PLANTS	ACTUAL MOISTURE AUG. 9	CONDITION OF PLANTS	ACTUAL MOISTURE SEPT. 10	CONDITION OF PLANTS	WATER REQUIREMENT
			per cent		*per cent*		*per cent*		*lbs.*
Clover	1	Min.	9.5	Firing	13.3	Dwarfed	17.8	Maturing	
	3	Min.	8.3	Firing	12.4	Dwarfed	16.3		
	3	Ave.	10.5	Wilting	17.3	Plump	16.5	Growing	
	4	Ave.	11.2	Wilting	16.2	Limp	18.5		
	5	Max.	12.4	Growth Checked	21.4	Thrifty	16.3		
	6	Max.	13.0		20.4	Thrifty	16.3		
Average			10.8		16.7		17.5		
Potatoes	7	Min.	12.4	Firing	14.5	Wilted	15.9	Maturing	497
	8	Min.	14.9	Checked	13.5	Wilted	14.0		260
	9	Ave.	24.9	Not Checked	19.0	Thrifty	17.2		278
	10	Ave.	20.0		15.3	Wilted	18.7	Growing	406
	11	Max.	20.5	Thrifty	16.9	Fair	17.7		456
	12	Max.	21.5	*Fallow*	18.0	*Fallow*	20.2		
Average			17.4		17.1		17.9		
Mangels	13	Min.	19.9	Limp	18.5	Wilted		Blighting	580
	14	Min.	19.9	Limp	14.2	Some fired			501
	15	Ave.	20.0	Thrifty	19.7	Some wilted			347
	16	Ave.	20.9	Thrifty	20.9	Plump			499
	17	Max.	26.5	Plump	21.7	Plump			486
	18	Max.	25.7	Plump	20.2	Plump			623
Average			20.4		20.3				
General Average			15.0		17.5		20.3		

In this table the humus content was determined by the ammonium method, and the moisture equivalent by the Briggs-Lane centrifuge or Briggs formula. In this table it appears that only the heaviest normal soils have as high a usable-water capacity as 2 inches per acre foot, these being silty clay loam, silt loam of high organic content. Peat, however, retains 3–4 inches of usable water per acre foot. The field capacity of usable water for silty loam soil is 1¾ inches; for sandy loam, 1¾–1¼ for fine sand, about 1 inch; for medium sand, about ⅔ inch; and for coarse sand as low as ½ inch per acre foot.

TABLE 3

Relation of soil type and usable water capacity to irrigation requirement

LOCATION	SOIL TYPE	HUMUS	HYGROSCOPIC MOISTURE	MOISTURE EQUIVALENT	MOISTURE CONTENT		TOTAL WATER-HOLDING CAPACITY		WEIGHT OF OVEN-DRY SOIL PER CUBIC FOOT	ESTIMATED EXCESS POINT	ESTIMATED DROUTH POINT	ESTIMATED USABLE-WATER CAPACITY	
					Mean maximum	Mean maximum							
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*in.*	*lbs.*	*per cent*	*per cent*	*per cent*	*in.*
Paisley	Medium peat	60.00			140	35	147	7.6	28	110	40	70	3.8
Paisley	Peaty silt loam	10.00	3.60	9.756	50	18	64	6.5	52	45	20	30	2.5
Paisley	Silt loam	7.60			40	16	50	5.8	63	34	16	18	2.1
Corvallis	Silty clay loam	5.50	3.75	10.16	30	12	36	5.5	80	27	14	13	2.0
Talent	Silty clay loam		3.53	9.57	24				75	22	10	12	1.7
Burns	Silt loam		5.24	14.20	45	20	36	4.9	66	35	18	17	2.1
Haines	Loam	7.65	2.60	7.05	29	12	38	4.0	68	27	12	15	2.0
LaGrande	Loam	*3.20	3.50	9.49	30	10				27	12	12	1.7
Burns	Very fine sand loam	2.49	5.78	15.66	40	17	38	4.6	64	33	18	15	1.8
Haines	Fine sandy loam	5.89	2.30	6.23	30	10	33	4.9	68	26	12	14	1.8
Grants Pass	Fine sandy loam	2.50			18	8	23	4.3	85	18	9	9	1.5
Joseph	Fine sandy loam	8.85	2.20	5.96	40	10	47	5.7	64	30	15	15	1.9
Paisley	Sandy loam	2.18	3.76	10.19	28	12	30	4.7	84	22	12	10	1.6
Redmond	Medium sand		3.50	9.49	30	12	39	4.1	66	24	12	13	1.6
Lakeview	Medium sand				27	10	28	4.5	84	20	11	9	1.5
Paisley	Coarse sand	2.65	2.26	6.12	15	5	21	3.5	85	14	7	7	1.1
Hermiston	Coarse sand	trace			12	6	18	3.0	88	11	7	4	0 7

* Determined by Brigg's formula instead of centrifuge.

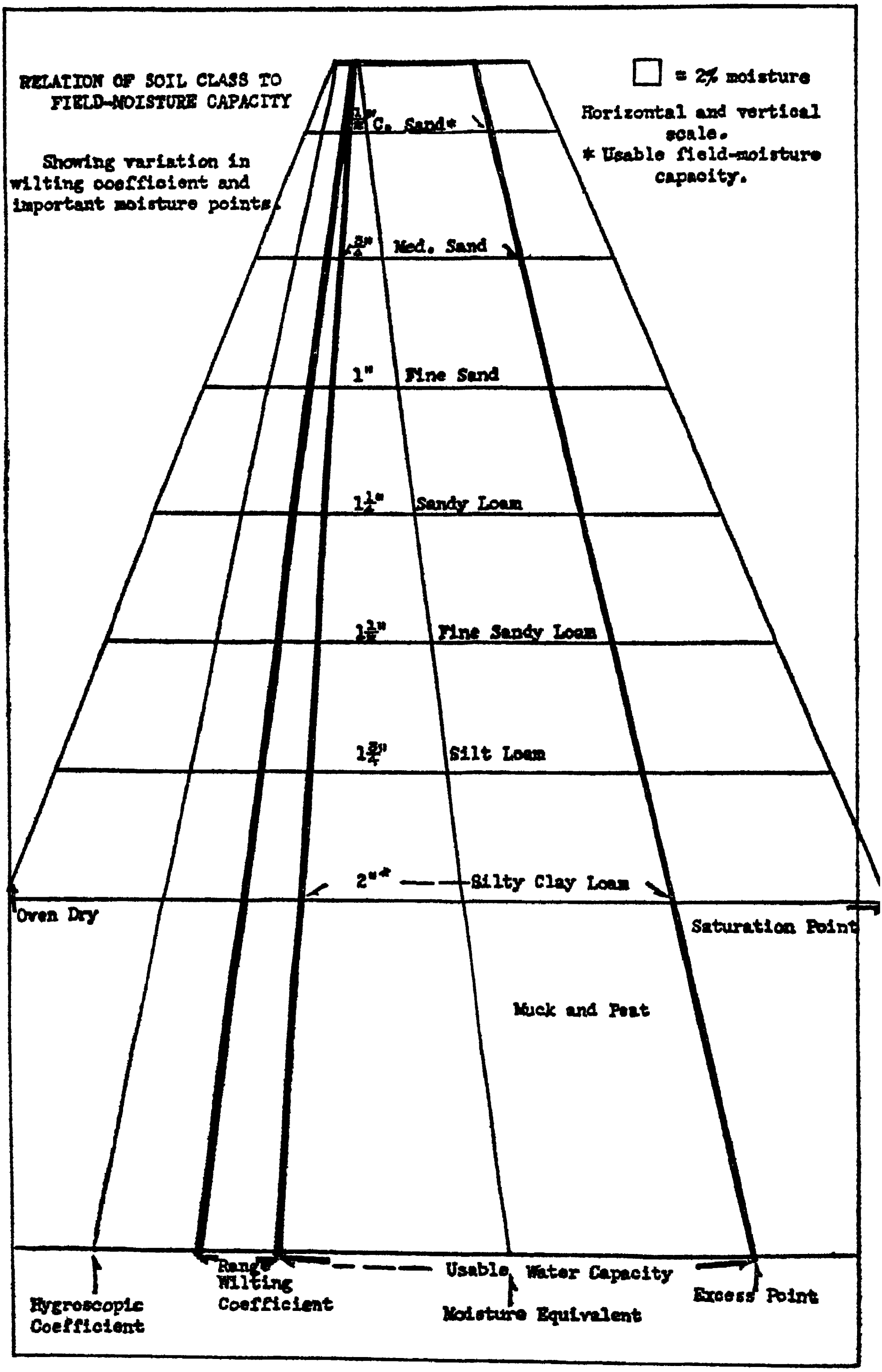

RELATION OF SOIL CLASS TO FIELD-MOISTURE CAPACITY
Showing variation in wilting coefficient and important moisture points.
□ = 2% moisture
Horizontal and vertical scale.
* Usable field-moisture capacity.
½" C. Sand*
¾" Med. Sand
1" Fine Sand
1¼" Sandy Loam
1½" Fine Sandy Loam
1¾" Silt Loam
2"* ——Silty Clay Loam
Oven Dry
Saturation Point
Muck and Peat
Hygroscopic Coefficient
Range Wilting Coefficient
Usable Water Capacity
Moisture Equivalent
Excess Point

FIGURE 1

SUMMARY

The wilting point or drouth point is a valuable indicator in connection with the determination of the exact moisture content at which to irrigate (the purpose of which is to maintain a favorable soil moisture content).

The wilting point varies more between different crops than has commonly been supposed when judged by field and tank studies of crop appearance, soil moisture and yields of dry matter.

The wilting point appears to vary more for different crops on a soil which is rather heavy in texture than on a soil of narrow moisture limits.

The wilting point marks the lower limit of usable water. Both affect the time and amount of irrigation.

The usable-field-moisture capacity as judged by samples taken before and after irrigation, cylinder tests of field moisture capacity, physical composition, and determination of other moisture points shows that only the heavier classes of normal soils are capable of retaining as much as two inches of usable water in the surface acre foot. The coarsest soils used for irrigation retain only half an inch per foot of depth while peat retains three or four inches of usable water per acre foot.

The irrigation requirement is greater for soils of coarse texture and low humus content and is largely due to unavoidable waste in connection with light frequent irrigation.

REFERENCES

(1) Briggs, L. J., and Shantz, H. L. 1912 The wilting coefficient of different plants and its indirect determination. U. S. Dept. Agr. Bur. Plant Indus. Bul. 230.

(2) Caldwell, J. S. 1913 The relation of environmental conditions to phenomenon of permanent wilting in plants. *In* Physiol. Res., vol. 1, pp. 1–56.

(3) Mosier, J. G., and Gustafson, A. F. 1917 Soil Physics and Management, pp. 212–213. Lippincott, Philadelphia.

(4) Powers, W. L. 1914 Irrigation and soil moisture investigations in western Oregon. Ore. Exp. Sta. Bul. 122.

(5) Powers, W. L. 1917 The economical use of irrigation water. Ore. Exp. Sta. Bul. 140.

OCCURRENCE OF SULFIDES IN MINNESOTA PEAT SOILS[1]

CLAYTON O. ROST
Minnesota Agricultural Experiment Station

INTRODUCTION

The common occurrence of iron sulfide in peat has long been recognized. As early as 1810, Rennie in classifying peats (4, p. 160), described "pyritous, or vitriolic peat" (4, p. 640) and mentioned several even earlier writers, including Brougmart (4, p. 640) and Bomare (4, p. 642), who had not only recognized pyrite in peat soils but had also observed its behavior upon coming into contact with the air and the resulting toxicity to crop plants. He quotes Bomare (4, p. 643) to the effect that "the ashes furnish an excellent manure, though the moss itself when applied as a top dressing, is *utterly destructive to vegetation.*" Wollny in 1897 (7, p. 231) mentions the occurrence of iron sulfide in peat as marcasite as well as pyrite.

Pyrite and marcasite are formed where water carrying some iron compound comes into contact with a solution of calcium sulfate, under conditions which favor the reducing action of plant residues (7, p. 231, 3, p. 100). The sulfides themselves, being insoluble in water, are not toxic, but upon contact with oxygen and water vapor are converted into ferrous sulfate and sulfuric acid, both of which strongly affect plants and, when present in large quantities, destroy all vegetation.

As the sulfides are the source of the toxic substances, the amount of these, their distribution in the soil profile, and relation to soil layers rich in lime are of chief interest in the present discussion. The quantities of ferrous sulfate and sulfuric acid found at any time will depend upon the amount of sulfides present and their distribution as well as upon the aeration and the drainage conditions. Ditching and tiling, while facilitating the removal of these harmful substances, at the same time increase the aeration and hasten the oxidation of the remaining sulfides. So under the influence of drainage alone the toxic compounds will not disappear until the whole of the sulfide has been oxidized and there has been sufficient movement of water through the soil to leach out the oxidation products (1, p. 63).

Both ferrous sulfate and sulfuric acid are rendered harmless when any form of agricultural lime, either the carbonate, oxide, or hydroxide, is mixed with the toxic layer. It is not uncommon, however, to find these substances at the bottom of bogs in which the peat at the surface and for some distance downward is well supplied with lime, and in the mineral substratum of which, only a few inches below, these is an abundance of carbonate. In some cases, also, the same layer contains both carbonate and pyrites, but under this circumstance the toxic oxidation products will be neutralized almost as rapidly as formed.

---

[1] Published with the approval of the Director as Paper No. 299, Journal Series of the Minnesota Agricultural Experiment Station.

In the reclamation of a bog the presence of a toxic layer becomes of importance since, if the peat is shallow, it may prevent the penetration of plant roots into the underlying mineral soil which otherwise might provide for any deficiencies of the peat in potash and phosphoric acid. Further, when material from such a toxic layer is thrown up in the course of ditching, and spread over adjacent land with the intention of improving it, it may prove injurious.

EXPERIMENTAL

In the autumn of 1918 several series of samples were collected from the Golden Valley Peat Experimental Fields in northern Minnesota. The chemical composition of the peat, the underlying muck and the mineral substratum is shown in table 1. In case of series A, B and C taken from the bank of a large drainage ditch adjacent to one of the experimental fields, it was found that the upper portion of the muck substratum and the section of peat immediately above it had a more strongly acid reaction, Truog method (6), than the layers above and below (table 2). From the surface down to this acid zone the peat was well supplied with lime, while the lowest part of the muck layer and the light-colored mineral substratum carried an abundance of calcium carbonate as shown by effervescence with dilute acid (table 2). Series D, taken from an excavation made in one of the experimental fields nearly a half mile back from the drainage ditch, did not show such marked differences in reaction.

The relative amounts of sulfide (table 2) were compared by using lead acetate paper. The procedure was briefly as follows:

A 10 gm. sample was placed in an Erlenmeyer flask of appropriate size, 100 cc. of distilled water and a few cubic centimeters of concentrated sulfuric acid added, a strip of filter paper moistened with lead acetate solution placed across the mouth of the flask and the contents of the flask brought to a boil and the boiling continued just 2 minutes. The degree of blackening of the lead acetate paper, or *sulfide coloration*, indicated the relative amounts of sulfide present which were designated by the same terms as are used on Truog's standard color chart (6, p. 8) to express the degrees of acidity.

In the levels nearest to the dividing line between the peat and the muck, the samples from the ditch bank showed a greater degree of acidity than corresponding samples taken from the excavation. There was, however, no difference in sulfide coloration. Along the bank of the ditch, which had been dug seven years before, some of the sulfide had been oxidized with consequent formation of sulfuric acid. This accounts for the more acid reaction of the sections carrying the larger amounts of sufide. In general the most sulfide

TABLE 1

Chemical composition of Golden Valley peat

DEPTH OF SECTION	WEIGHT PER CUBIC FOOT	LOSS ON IGNITION	ASH	NITROGEN	LIME (CaO)	PHOSPHORIC ACID	REACTION BY TRUOG METHOD
in.	*lbs.*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	
0–6	7.9	87.0	13.0	2.43	2.05	0.18	Very slight
7–12	9.9	80.0	20.0	2.46	3.29	0.20	
13–18	9.9	89.2	10.8	2.56	2.35	0.14	
19–24	12.6	88.2	11.8	2.57	2.39	0.15	Slight
25–30	13.9	87.4	12.6	2.96	2.21	0.15	Strong
31–33		32.8	67.2	1.23	2.12	0.14	Very strong
34–36		6.8	93.2	0.238	3.05		Neutral
37–39				0.163	8.99		
40–42				0.120	12.15		

TABLE 2

Reaction and relative amounts of sulfide in successive levels

Samples of 1918*

DEPTH	SAMPLES FROM DITCH BANK						SAMPLES FROM EXCAVATION	
	Series A		Series B		Series C		Series D	
	Acidity	Sulfide coloration	Acidity	Sulfide coloration	Acidity	Sulfide coloration	Acidity	Sulfide coloration
in.								
0–6	v. sl†	sl	v .sl	sl	v. sl	v. sl	v. sl	sl
7–12						v. sl	v. sl	sl
13–18						med	sl	med
19–24	sl	med	v. sl	med	sl	sl	v. sl	med
25–27	str	str	v. sl	med	sl	med	v. sl	sl
28–30					v. sl	v. sl	neut	sl (e)
31–33	v. str	med	sl	med	neut	sl (e)	neut	sl (e)
34–36	neut	sl (e)	sl	med	neut	v. sl (e)	neut	v. sl (e)
37–39			v. sl	sl	neut	v. sl (e)	neut	v. sl (e)
40–42			sl	sl	neut	v. sl (e)	neut	v. sl (e)
43–45	neut	....	sl	sl	....	....	neut	none
46–48	neut	....	neut	sl	....	....	....	....
49–51	....	....	neut	sl (e)	....	....	....	....
52–54	....	....	neut	v. sl (e)	....	....	....	....
55–57	....	....	neut	....	....	....	....	....

* Determinations were made 2 months after the samples were collected. The heavy cross lines indicate the approximate boundary between the peat layer and the underlying mineral soil.

† neut = neutral, v. sl = very slight, sl = slight, med = medium, str = strong, v. str = very strong, e = effervesces with dilute acid.

was found in the layer of peat immediately above the muck and not in the muck layer itself, just the opposite of Ramann's conclusion as to European peats. Ramann considered the sulfide more common in the muck layer underlying the peat than in the peat itself (3, p. 100).

In the samples dealt with, the sulfide occurred in such a finely divided state that it was not visible to the naked eye, and could be identified only by chemical tests. Typical samples showed the presence of iron while the two that were most strongly acid, those from the 28–30 and 31–33 inch sections of Series A, showed the presence of ferrous iron.

Two areas in the northwestern and one area in the northeastern part of the state were examined for sulfides and acidity during the season of 1919. Samples were collected only from the six-inch layer of peat immediately above the muck and from the upper portion of the muck layer. One of the northwestern areas extended from Golden Valley eastward some 6 miles and included the Golden Valley Peat Experimental Fields, of which a thorough examination was made, while the other, some 25 miles southeast of the first, embraced an area of shallow peat to the east of Thief River Falls. The one in the northeastern part of the state, was in the vicinity of Meadowlands, in St. Louis County, embracing an area 15 by 5 miles. In order to avoid any oxidation of the sulfide between the time of taking the samples and their laboratory examination, a field outfit was carried and the samples tested within a few hours after being taken. When shipped and later stored at the laboratory for some time before testing, samples had been found to show a more strongly acid reaction and less sulfide coloration.

Of the 20 series of samples taken from the Experimental Fields at Golden Valley in the second season, only one showed a strongly acid reaction in the peat layer, while all responded to the test for sulfides (table 3). Also the muck showed less acidity and less sulfide than the peat and in the case of three of the muck series, both acidity and sulfide were absent. This difference from the results of the first season is to be attributed to a flood (2, p. 51) following a torrential rain in the early part of July. Erosion of the walls of the large drainage ditch from which samples had been taken the year before widened it some 12 or 15 feet and exposed fresh sections on both sides. Samples taken from freshly exposed portions showed the same general condition as found farther back. There was no markedly acid peat or muck layer (table 3), and the sulfide coloration was about the same as the year before.

The samples taken at one-mile intervals east of Golden Valley and those taken east of Thief River Falls very closely resembled those taken on the Experimental Fields, in both reaction and sulfide content. Thus in general the results were very similar to those secured in 1918 with samples taken from the excavation on the experimental tract.

In only one place in the Meadowlands area was sulfide found. This was in a small "pocket" in which there was an unusual depth of muck—about two feet. In this instance the sulfide was more abundant in the uppermost portion of the muck layer than in the peat. A summary of the samples tested and the number of these showing a sulfide coloration is given in table 4.

During the field work of the second season a set of 19 samples was collected for chemical analysis. These consisted of nine pairs of samples made up of the upper 6-inch layer of muck substratum and the 6-inch layer of peat immediately above this, taken at different places on the Experimental Fields at Golden

TABLE 3

Reaction and relative amounts of sulfide in peat soil from northwestern Minnesota

Samples of 1919

SERIES	PEAT		MUCK	
	Acidity	Sulfide coloration	Acidity	Sulfide coloration
Golden Valley Peat Experiment Field				
1	v. sl	sl	neut	v. sl
2	v. sl	v. sl		sl
3	str	sl	sl	sl
4	sl	v. sl		none
5	sl	sl		v. sl
6	sl	v. sl		none
7	v. sl	sl	v. sl	none
8	v. sl	sl		v. sl
9	v. sl	sl		v. sl
10	v sl	sl		v sl
11	v. sl	sl		v. sl
12	v. sl	sl	neut	v. sl
13	neut	sl		sl
14	neut	sl		sl
15	v. sl	sl		v. sl
16	v. sl	sl		v. sl
17	v. sl	sl		v. sl
18	neut	sl	neut	v. sl
19	neut	med	v. sl	sl
20	v. sl	sl		sl
Ditch Bank at Golden Valley				
21	v. sl	v. sl		v. sl
22	v. sl	v. sl	sl	v. sl
23	v. sl	v. sl		none
24	v. sl	v. sl		v. sl
25	v. sl	v. sl		sl
East of Golden Valley				
26	v. sl	sl		sl
27	v. sl	sl		v. sl
28	neut	sl		v. sl
29	neut	sl		v. sl
30	v. sl	sl		sl
31	neut	v. sl		none
Near Thief River Falls				
32	sl	sl	neut	v. sl
33	sl	sl	neut	v. sl
34	sl	sl	neut	v. sl
35	neut	sl	neut	v. sl

TABLE 4

Number of samples showing the presence of sulfides

Samples of 1919

AREA	PEAT		MUCK	
	Number of samples collected	Number showing sulfide	Number of samples collected	Number showing sulfide
Golden Valley	50	50	51	46
Thief River Falls	4	4	4	4
Meadowlands	18	1	18	1

Valley, and of one sample of peat taken 4 miles southeast of Thief River Falls. To insure the exclusion of any air and the consequent oxidation of the sulfide present, the samples were packed firmly into 2-quart tin cans, tightly covered and sealed with paraffin. When the samples were opened in the laboratory the seals on all were found to be in good condition. The acidity and the sulfide coloration, as well as the amount of sulfide, expressed as H_2S, are reported in table 5. The iodine method (5, p. 398) used in determining the last is briefly as follows:

Twenty-five grams of moist peat or 50 gm. of muck is weighed into a 500-cc. Erlenmeyer flask, 100 cc. of distilled water and 10 cc. of stannous chloride added. The flask is then attached to a condenser in turn connected with another flask containing an ammoniacal solution of cadmium sulfate. Twenty cc. of hydrochloric acid is added to the contents of the flask through a separatory funnel and the whole brought to boiling and then gently boiled for 20 minutes. The contents of the flask containing cadmium sulfide are then nearly neutralized with hydrochloric acid and titrated with thirtieth normal iodine in the usual manner.

TABLE 5

Sulfide, reaction, and sulfide coloration of 19 samples of peat and muck

SAMPLE	PEAT			MUCK		
	Reaction	Sulfide coloration	Sulfide*	Reaction	Sulfide coloration	Sulfide
			per cent			*per cent*
4	sl	med	0 060	str	sl	0.006
1	str	med	0.058	neut	v. sl	0.002
5	v. sl	med	0.047	med	sl	0.014
3	sl	sl	0.037	neut	v. sl	0.005
9	sl	sl	0.036	str	v. sl	0.004
8	sl	sl	0.032	sl	v. sl	0.002
2	sl	sl	0.028	neut	v. sl	0.003
6	str	sl	0.025	neut	v. sl	0.003
7	v. sl	sl	0.016	v. sl	v. sl	0.002
10	sl	sl	0.016			

* Expressed as H_3S.

With both the peat and the muck there is a general agreement between the coloration of the lead acetate paper and the amount of sulfide found, i.e., the darker the sulfide coloration the larger is the amount of sulfide found. However, when a peat and a muck which show the same degree of sulfide coloration, are compared, no direct relation is shown between the degree of coloration and the amount of sulfide. The peat layer contains the larger amounts of the latter, varying between 0.016 and 0.060 per cent as compared with 0.002 to 0.014 per cent in the muck, and, for any given degree of sulfide coloration, shows a higher actual content of sulfide.

No concordance was found between the degree of acidity and either the sulfide coloration or the amount of sulfide the acidity being governed largely by conditions favoring oxidation and by the presence of carbonate.

Crops on the untreated plots on the Golden Valley field were comparative failures, with the exception of flax (2, p. 33), but when given a dressing of acid phosphate they yielded as well as on the surrounding mineral soil. On properly fertilized plots near the places where samples 1, 2, 5 and 6 were taken, the living plant roots were found to penetrate both the lowermost layer of peat and the muck, and even to extend down into the mineral subsoil. Thus either the amount of sulfide oxidized must be regarded as too small to harm the growing plants or the carbonate present neutralized most of the acid as rapidly as formed.

In a vegetation experiment with a pyrite-carrying muck from a shallow bog near Goodridge, 15 miles southeast of Golden Valley, clover plants on the untreated muck watered with distilled water made almost no growth, although at the end of a year were still alive. On the same muck treated with the ash obtained by burning a portion of the overlying peat and watered with tap water carrying large amounts of lime the growth of clover was excellent, the calcium carbonate in the ash and water being sufficient to render the two toxic compounds harmless. In the same Goodridge field complete reclamation was effected by the application of acid phosphate (1, p. 63).

In a similar vegetation experiment carried out in the plant house a year later with untreated muck from the Golden Valley Experimental Fields sweet clover did well when watered freely with the tap water.

On the surface of the peat in northwestern Minnesota, gypsum often occurs as a white incrustation sometimes so heavy that it crackles under the foot. It is also always to be found on the faces of ditches. A sample of the material intermixed with more or less peat from Golden Valley was subjected to repeated extractions with warm water until the leachings failed to give a test for sulfates, the extract evaporated on the water bath, and analyzed with the following results:

Fe_2O_3	0.0 per cent
Al_2O_3	0.0 per cent
CaO	28.5 per cent
MgO	4.1 per cent
So_3	55.5 per cent
Total Solids	0.1015 gram

SUMMARY

In samples of peat and muck from the Golden Valley Peat Experimental Fields in northwestern Minnesota in 1918, sulfides were generally found at all levels in the peat, in the muck substratum and in the upper portion of the mineral subsoil below. The greatest amount was found in the lowest portion of the peat layer and not in the muck.

The reaction of the peat and muck was found to be but little related to the relative amount of sulfides present, but to conditions permitting the oxidation of the sulfide to sulfuric acid and ferrous sulfate.

The layers in an exposed ditch face strongly impregnated with sulfide gave in 1918 a more acid reaction than did those at some distance from the ditch. But a year later, just after an unusual flood had widened the ditch and exposed fresh material, the peat of the ditch face was found to be similar in reaction to the corresponding layers farther back.

Expressing the sulfide content as hydrogen sulfide, nineteen samples of peat and muck showed a maximum of 0 060 and a minimum of 0.016 per cent for the lowermost layer of peat and 0.013 and 0.002 per cent for the muck substratum immediately below.

Sulfides appear to be much more commonly associated with peat in northwestern than in northeastern Minnesota, where in an area of approximately 75 square miles, sulfides were found at only one place.

REFERENCES

(1) Alway, F. J. 1920 Agricultural value and reclamation of Minnesota peat soils. Minn. Agr. Exp. Sta. Bul. 188.

(2) Alway, F. J. 1920 Report of Golden Valley Peat Experimental Fields, 1918 and 1919. Minn. Agr. Exp. Sta. Bul. 194.

(3) Ramann, E 1911 Bodenkunde, 3d ed., Julius Springer, Berlin.

(4) Rennie, Rev. R. 1810 Essays on the Natural History and Origin of Peat Moss. Edinburgh.

(5) Scott, W. W 1917 Standard Methods of Chemical Analysis. D. van Norstrand Co., New York.

(6) Truog, E. 1915 A new test for soil acidity. Wis. Agr. Exp. Sta. Bul. 249.

(7) Wollny, 1897 Die Zersetzung der organishen Stoffe und die Humusbildungen. Carl Winter, Heidelberg.

THE HUMAN ELEMENT IN THE WORK AT ROTHAMSTED

Reprinted from the Agricultural Gazette

It was a happy idea that led Mr. Edwin Grey, field superintendent at the Rothamsted Experimental Station, to write his reminiscences. They cover a period of fifty years. Since his first job at the laboratory, as a boy of thirteen, was in the summer of 1872, he was working there for nearly fifty years in the lifetime of Lawes and Gilbert. Of the men who carried on field operations under the eyes of those two great leaders, we believe he is now the only survivor. His book will serve to keep green the memory of many who helped carry out the work which the founders of the Station planned.

Mr. Grey has produced a very interesting book, none the less so in that it is altogether free from the stiffness and formality of the regular author. He lets his pen run on just as we can imagine his tongue might do, in an easy, unreserved moment, recalling what he has witnessed—the oxen ploughing in the Broadbalk Field, before all the work was done by horses, to give place still later to the tractor—the Harvest Home suppers in the "East Mow"—the Irish laborers—the Christmastide presents—the casuals discussing the merits of "His Majesty's Hotel" for the winter—the Jubilee festivities—the theatricals in the "Sample House." He gives us glimpses of a rural life which has altogether vanished, thumbnail sketches of countryside characters which the changes of the last twenty or thirty years have made as obsolete as the very dodo—the old bird scarer who resented Sir John Lawes' fondness for a rookery, shouting to the birds, "Go back to Rothamsted, you old black devils, and let Jack Lawes look arter yer!"—the man who, as the nights drew in, remarked "That'll get late proper early tonight, I'll warrant,"—the boy who entered in his record book, "Through there being no cake, the cows got only half a lot."

Sir Henry Gilbert is shown as a very human, kindly, if occasionally an irascible old gentleman, losing his temper over misunderstood instructions and being quieted down by Lady Gilbert; or telling the man who thought that the water samples should be brought down in a cart drawn by 'an experimental donkey,' "Well, Frank, we've already got one or two about here now." Sir John Lawes too, appears and reappears throughout the volume as the generous squire, as well as the far-seeing man of science. But it is as a record of humbler lives that the book has its greatest charm. It gives us the human side of what countless papers have given the scientific results; and throughout its pages we are shown the patient toil, the cheerful acceptance of a life of hard work, the patience, the honesty, and good faith of the men, who in their own way had their share in the operations which are inseparably bound up with the name of Rothamsted.

Sir E. J. Russell has written a brief preface, and the book is illustrated with a number of photographs and sketches; but it is the author's straight forward story, revealing quite unconsciously his tact and sympathy, and illuminated by a quiet humor, which is the mainstay of a very delightful volume. The book can be obtained post free for 5s. 9d. from E. Grey, Laboratory Cottages, Harpenden, or from the Secretary, Rothamsted Experimental Station, Harpenden, Herts.

VARIABILITY OF ALKALI SOIL[1]

W. P. KELLEY[2]

Citrus Experiment Station, University of California

Received for publication November 20, 1921

Waynick (5) found that the nitrate content of soil samples drawn from a small area of apparently uniform soil, and especially the amounts of nitrate formed in the samples during a period of four weeks, varied quite widely. Waynick and Sharp (6) found considerable differences in both the nitrogen and the carbon content of soil samples taken at 30-foot intervals from two different areas of apparently uniform soil. McBeth (3) showed that great variability characterizes the distribution of nitrate in the soils of several citrus groves in California. Similar results have been observed by this laboratory.

Those who are familiar with semi-arid regions are quite aware of the sporadic variability of alkali soils. Not infrequently abrupt differences occur in the growth of crops. Good yields may be obtained in a given location, while only a few feet distant neither growth nor germination is possible. The lack of uniformity in the salt content of the soil and other factors (4), some of which will be discussed later, greatly complicate plot experimentation on alkali soils and render it extremely difficult to secure uniform results by the application of a given treatment. As is shown below, the usual method of sampling alkali soil may be quite unreliable.

EXPERIMENTAL PLOTS

In connection with an alkali reclamation experiment consisting of approximately seven acres, and located on a quarter section of the Kearney Ranch near Fresno, California, a large number of soil samples have been analyzed. The experiment was begun in May 1920, just previous to harvesting that season's barley crop. The soil appeared to be as nearly uniform as could be found anywhere within the entire quarter section. The barley crop of 1920 was a complete failure on the greater portion of the area. On much of it the seed had failed to germinate, although the moisture and other seasonal conditions were quite favorable for growth. There were several small spots within this area, however, on which the barley made slight growth, and on a few spots a reasonably good yield was obtained.

---

[1] Paper No. 86, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

[2] Analyses reported were made by S. M. Brown, Assistant in Chemistry, at the Citrus Experiment Station.

The experiment consists of 9 plots 100 feet by 330 feet in size. After making an accurate map showing the barley and weed growth, a line was drawn across the greatest length of each plot, and soil samples were taken on this line, usually at intervals of ten feet. The samples were drawn with a two-inch auger and were taken to represent the first-foot and second-foot depths respectively, and in a considerable number of instances the third-foot and fourth-foot depths also.

Extracts were prepared by shaking a portion of each sample for one hour with distilled water in the ratio of 1:5 and filtering the solutions through Pasteur-Chamberland tubes. The extracts were analyzed by methods previously described (1).

RESULTS

As was suggested by the appearance of the barley crop, the analyses show that this soil is distinctly spotted. Every sample from certain spots was substantially different in composition from those taken from other spots. A few of these spots cannot be classified as alkali soil, and certain other spots contain amounts of salts quite different from those of still other spots. The results also show that the distribution of the various salts within spots of apparently uniform soil is extremely variable. The analyses indicate that at least two factors must be taken into consideration in studying the variability of this soil, (a) variation within the several spots and (b), the nature of a given spot as a whole, that is, whether alkali salts are present or absent,[3] and if present, whether the general level of concentration is high or low, etc.

The soluble constituents of those samples which gave a distinct reaction with phenolphthalein were composed principally of sodium salts, but wherever sodium carbonate was absent the amounts of soluble alkaline earths, especially calcium, were substantially greater than in the more highly alkaline spots.

The anions common to alkali soil, the total soluble salts and the pH values of the samples representing the first-foot and second-foot sections of only two plots will be presented here. These plots are fairly representative of the area as a whole. Their analyses are presented in tables 1, 2, 3 and 4.

The data show that this soil varies greatly in composition. In one instance the chloride content of two samples taken ten feet apart differed almost 1000 per cent, and in several other instances differences as great as 100 per cent, or more, were found. The content of the different constituents does not necessarily vary in the same direction. For example, certain samples contained sub-

---

[3] Because of the complex nature of the variation in this soil the writer does not consider it permissible to apply the usual statistical treatment to the data now at hand. For instance, it was found that the frequency curve obtained by plotting the chlorine of plot 3 bears very little similarity to a theoretical frequency curve. As pointed out by Linhart (2) the usual statistical formula is not applicable to data which give curves widely divergent from the theoretical curve. It should not be inferred, however, that statistical methods cannot be applied to the study of spotted soil. It is not entirely clear to the writer, however, that there is any particular advantage to be derived from a determination of the mean composition of spotted soil.

TABLE 1

*Composition of soil samples, plot 3, first foot**

SAMPLE NUMBER	1	2	3	4	5	6	7	8	9	10	11	12	13	14
	Parts per million													
CO_3	435	375	330	330	285	315	210	330	210	240	120	60	90	90
HCO_3	412	412	336	335	396	412	534	472	472	488	366	427	442	335
Cl	913	629	727	140	124	195	629	426	204	167	53	53	35	44
SO_4	474	376	588	70	62		110	129	151	68	39	43	27	29
NO_3	462	201	201	62	62	46	139	15	31	62	62	46	46	62
Total salts	4525	3235	3360	1565	1570	1790	2795	2430	1460	1605	990	1050	940	740
pH value	9.6	9.6	9.7	9.6	9.6	9.6	9.6	9.6	9.6	9.6	9.0	9.2	9.3	9.2

SAMPLE NUMBER	15	16	17	18	19	20	21	22	23	24	25	26	27	28
	Parts per million													
CO_3	195	75	240	225	330	390	270	90	75	105	390	375	450	465
HCO_3	442	320	351	564	366	381	396	351	320	351	457	640	488	442
Cl	26	44	44	655	230	362	265	18	35	35	185	133	106	576
SO_4		22	31	88	130	149	103	21	18	27	110	33	266	241
NO_3	31	93	62	124	231	200	77	46	93	62	93	308	201	263
Total salts	1005	660	1140	1770	2280	2675	1750	805	765	930	2220	3460	3060	3285
pH value	9.6	9.2	9.4	9.5	9.6	9.7	9.6	9.2	9.2	9.2	9.6	9.6	9.6	9.6

* The distance between samples 13 and 14, 15 and 16, 23 and 24, and 24 and 25, was 20 feet. All other samples were taken 10 feet apart.

TABLE 2

Composition of soil samples, plot 3, second foot

SAMPLE NUMBER	1	2	3	4	5	6	7	8	9	10	11	12	13	14
	Parts per million													
CO_3	120	45	75	15	120	105	120	180	150	150	150	120	105	90
HCO_3	442	549	534	472	320	305	335	351	335	229	366	274	305	320
Cl	213	284	364	638	603	825	541	266	222	310	177	124	53	44
SO_4	198	110	130	414	278	387	165	177	119	119	113	68	68	75
NO_3	108	93	108	186	108	62	31	31	0	0	4	0	2	0
Total salts	1490	1465	1625	2570	2265	2710	2210	1500	1375	1515	1260	810	750	735
pH value	9.6	9.4	9.6	9.2	9.3	9.2	9.4	9.6	9.6	9.6	9.4	9.4	9.6	9.4

SAMPLE NUMBER	15	16	17	18	19	20	21	22	23	24	25	26	27	28
	Parts per million													
CO_3	120	30	120	90	75	150	90	45	45	120	210	300	150	150
HCO_3	351	259	503	457	274	335	488	351	274	335	488	488	303	335
Cl	35	27	142	248	151	266	426	53	27	80	248	177	284	337
SO_4	66	59	56	108	79	93	179	33	39	44	160	168	183	160
NO_3	0	2	62	155	31	93	31	0	0	0	46	46	31	31
Total salts	740	610	1150	1500	1200	1500	1725	620	670	875	1805	2015	1430	2015
pH value	9.6	8.6	9.6	9.6	9.0	9.8	9.4	9.0	8.8	9.6	9.7	9.8	9.4	9.6

TABLE 3

*Composition of soil samples, plot 8, first foot**

SAMPLE NUMBER	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	Parts per million														
CO_3	210	330	330	330	405	405	420	240	75	0	0	0	0	0	0
HCO_3	381	244	366	412	397	397	488	458	503	275	290	275	198	107	107
Cl	894	220	108	354	1133	1070	327	53	18	18	18	18	18	18	18
SO_4	273	117	42	149	537	491	195	66	39	29	26	22	26	18	20
NO_3	90	45	75	90	300	270	150	60	0	0	0	0	0	0	4
Total salts	3105	1650	1585	2360	5000	4550	2620	1265	750	400	475	350	340	255	250
pH value	9.7	9.4	9.7	9.6	9.7	9.8	9.8	9.4	9.2	7.8	7.6	7.6	7.6	7.2	7.4

SAMPLE NUMBER	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
	Parts per million														
CO_3	0	0	0	0	0	0	0	0	0	0	150	210	300	315	435
HCO_3	229	275	275	260	275	229	214	214	229	260	473	564	350	458	412
Cl	18	18	18	18	18	18	18	18	18	18	62	45	53	140	168
SO_4	18	21	23	19	22	25	22	20	39	31	54	37	58	132	606
NO_3	4	0	3	0	3	4	0	4	0	20	15	60	60	75	165
Total salts	280	350	355	365	515	470	540	525	500	550	1120	1050	1365	3275	2050
pH value	7.6	8.0	7.8	7.8	8.1	7.6	7.6	7.6	7.8	8.2	9.6	9.6	9.7	9.6	9.8

* Distance between samples 16 and 17 was 30 feet. All other samples were taken 10 feet apart.

TABLE 4

Composition of soil samples, plot 8, second foot

SAMPLE NUMBER	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	Parts per million														
CO_3	15	0	75	120	75	120	285	390	195	60	0	0	0	0	0
HCO_3	199	244	366	244	199	199	214	259	290	305	214	153	153	92	122
Cl	798	1283	691	840	363	470	372	204	80	71	18	89	27	53	35
SO_4	258	428	264	313	126	216	160	124	24	31	28	24	33	25	23
NO_3	0	9	16	9	18	46	46	15	5	0	0	2	2	0	0
Total salts	2090	3000	2090	2085	1280	1850	2055	2050	1000	670	425	400	250	255	255
pH value	8.4	...	9.2	9.4	9.4	9.6	9.6	9.6	9.6	9.4	8.0	7.8	7.8	8.0	7.8

SAMPLE NUMBER	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
	Parts per million														
CO_3	0	0	0	0	15	0	0	0	0	30	150	105	180	150	90
HCO_3	153	106	229	199	381	198	153	168	183	305	244	351	274	229	214
Cl	44	44	27	62	35	124	35	53	35	53	115	160	363	284	230
SO_4	25	32	31	33	30	41	23	33	18	24	82	103	271	242	140
NO_3	0	0	0	0	4	5	0	0	3	9	46	18	18	31	9
Total salts	300	300	275	255	225	215	230	220	290	490	1600	1210	1685	1500	715
pH value	7.8	7.8	8.0	7.6	8.8	7.6	7.8	7.8	7.6	8.6	9.6	9.6	9.6	9.6	9.0

stantially greater amounts of chloride than of any other ion, while other samples contained greater amounts of normal carbonate, sulphate or nitrate than of chloride.

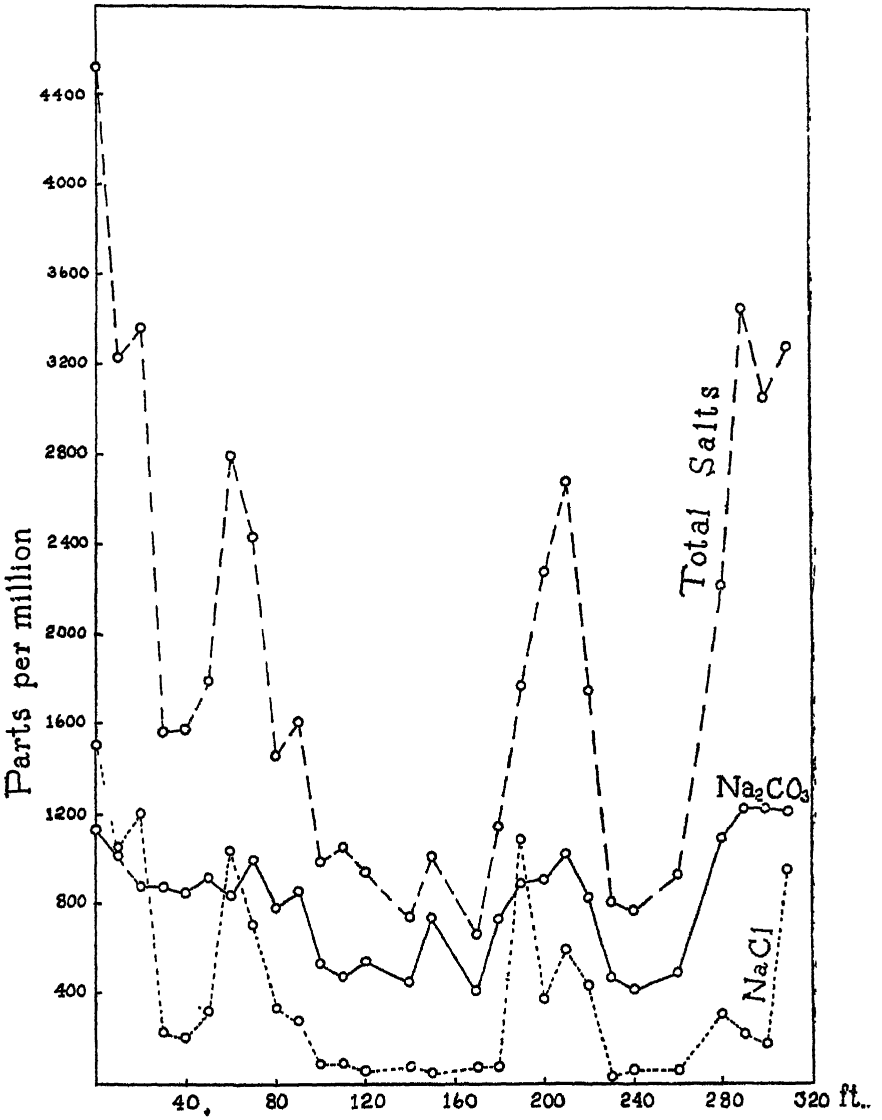


FIG. 1. GRAPHS SHOWING THE LINEAR VARIATION OF AN ALKALI SOIL, FIRST FOOT, PLOT 3. Na_2CO_3 calculated from methyl orange titrations

On that portion of plot 3 from which samples 1 to 9 inclusive were taken, the barley crop was a complete failure. Among these samples, 4, 5 and 6, representing the first foot, contained quantities of chloride, sulfate and nitrate, each of which is certainly below the limits of toxicity, but here, as is the case with the larger portion of this area, toxic concentrations of sodium carbonate occur.

A sparse stand of barley, varying from 10 to 30 inches in height, was obtained on those portions of plot 3 from which samples 9 to 16 and 22 to 24 inclusive, were taken. It is interesting to note that these samples contained considerably lower concentrations of salts than the other samples from this plot. It is quite probable that the concentration of sodium carbonate present in these spots was sufficiently high to be injurious, although not so high as entirely to prevent the growth of barley.

The portions of plot 8 from which samples 1 to 8 and 26 to 30 inclusive were drawn, were entirely destitute of vegetation. It will be noted that, while all of these samples contained relatively high concentrations of sodium carbonate, several of them contained rather low concentrations of the neutral salts.

The portion of plot 8 from which samples 9 to 25 inclusive were drawn is not an alkali soil. None of the samples from it contained an excess of any salt, although this small area is entirely surrounded by highly saline or alkaline soil. Although this spot is practically free from alkali salts, it produced a very low yield of barley. It will be shown subsequently that the inferiority of the barley here was due to the severity of the competition with several different species of weeds.

The samples representing the second foot were usually found to contain lower concentrations of salts than those of the first foot. In a few cases, however, greater amounts of chloride and sulphate were found than in the first foot. With only a few exceptions, the samples representing the third and fourth feet contained still lower concentrations of salts than the corresponding samples of the second foot.

The almost complete absence of nitrate in certain places was probably due, in part at least, to the fact that the samples were taken in close proximity to growing barley or weeds.

The data as a whole indicate that sodium carbonate is by far the most important salt in this soil.

Samples from specially chosen locations

In order to study the variability of other portions of this quarter section, three additional series of samples were taken in April, 1921. Two of these, series A and B, were drawn from a line of holes extending perpendicularly across the margins of alkali spots, a part of each series being taken from barren soil and a part from fairly productive soil. The distance between the places of sampling was either 2 or 3 feet. The analysis of series A and B is recorded in tables 5, 6, 7 and 8.

The composition of the samples composing series A varied quite widely. Among those representing the first foot, sample 3 contained more than three

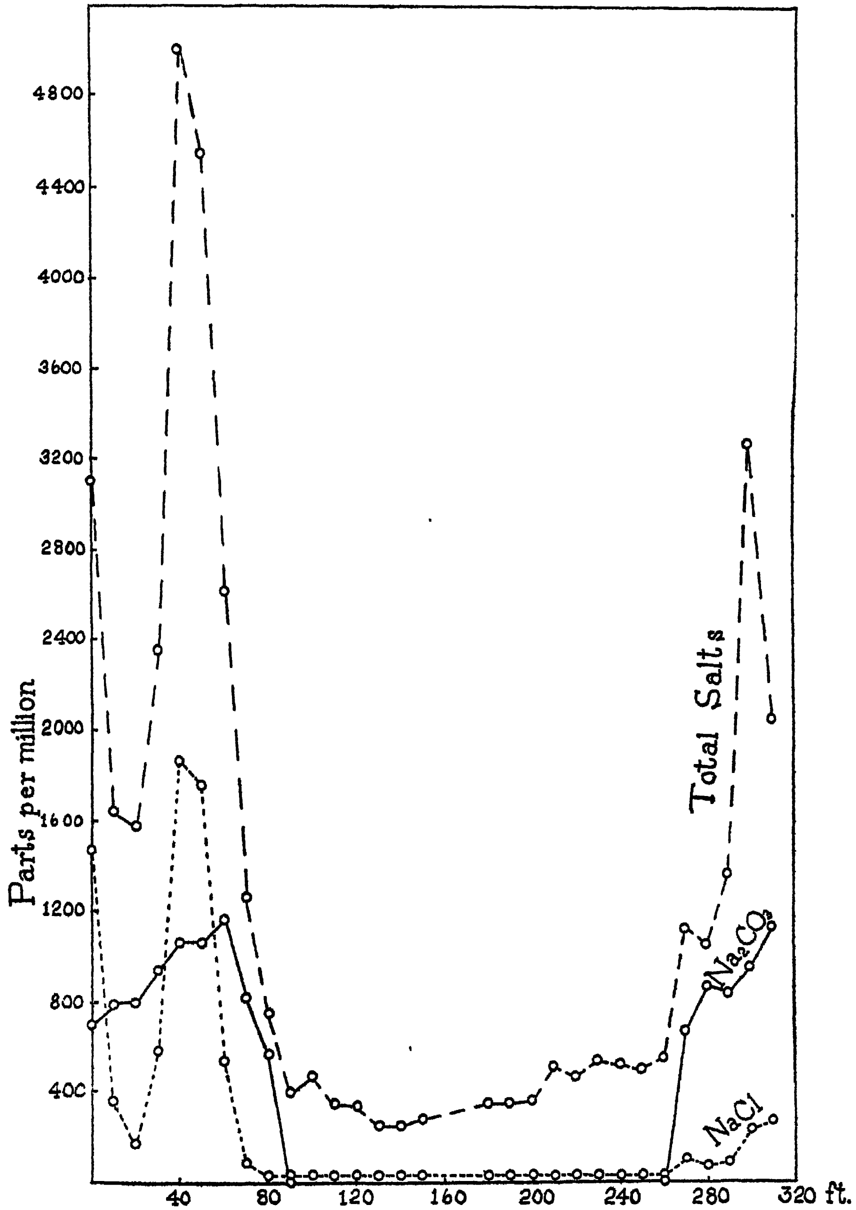


FIG. 2. GRAPHS SHOWING THE LINEAR VARIATION OF AN ALKALI SOIL, FIRST FOOT, PLOT 8

Na_2CO_3 calculated from methyl orange titrations unless extract failed to give a reaction with phenolphthalein and also contained a substantial amount of calcium.

TABLE 5

Composition of soil samples, series A, first foot

SAMPLE NUMBER AND DISTANCES	1	2 3 FT. FROM 1	3 3 FT. FROM 2	4 2 FT. FROM 3	5 2 FT. FROM 4	6 2 FT. FROM 5	7 2 FT. FROM 6	8 2 FT. FROM 7	9 2 FT. FROM 8
Growth of barley	None	None	None	None	Slight stand, 12 in. high	Thin stand, 18 in. high	Fair stand, 30 in. high	Good stand, 36 in. high	Good stand, 36 in. high
	Parts per million								
CO_3	420	300	255	270	270	210	0	0	0
HCO_3	320	366	381	412	259	259	335	335	320
Cl	355	514	798	222	168	133	496	115	62
SO_4	202	303	443	61	62	13	170	110	11
NO_3	89	62	7	4	0	0	9	0	0
Total salts	2400	2700	3240	1680	1355	1035	1450	1125	735
pH value	9.6	9.6	9.5	9.6	9.6	9.6	7.3	7.7	8.2

TABLE 6

Composition of soil samples, series A, second foot

SAMPLE NUMBER	1	2	3	4	5	6	7	8	9
	Parts per million								
CO_3	150	135	150	75	180	180	0	15	15
HCO_3	183	229	244	274	229	214	442	305	290
Cl	177	186	390	106	177	142	213	248	97
SO_4	37	52	179	33	43	16	67	90	13
NO_3	19	18	5	4	0	0	9	0	0
Total salts	1000	1085	1665	720	1155	950	910	1150	710
pH value	9.4	9.3	9.4	9.2	9.6	9.6	8.0	8.6	8.8

TABLE 7

Composition of soil samples, series B, first foot

SAMPLE NUMBER AND DISTANCES	1	2 3 FT. FROM 1	3 3 FT. FROM 2	4 2 FT. FROM 3	5 2 FT. FROM 4	6 2 FT. FROM 5	7 2 FT. FROM 6	8 3 FT. FROM 7
Growth of barley	None	None	None	None	Slight stand, 12 in. high	Thin stand, 24 in. high	Good stand, 30 in. high	Good stand, 36 in. high
	Parts per million							
CO_3	285	270	300	195	90	90	45	30
HCO_3	274	366	290	335	473	366	442	412
Cl	44	62	35	71	97	160	168	133
SO_4	67	64	73	62	31	69	43	134
NO_3	0	5	7	5	9	4	0	5
Total salts	1100	1200	1075	970	1180	1100	925	1000
pH value	9.6	9.6	9.6	9.6	9.2	9.2	9.1	9.0

times as much chloride as sample 4 taken two feet distant, and number 7, contained approximately 4 times as much chloride as number 8. It will be noted that considerable amounts of sodium carbonate were found in each sample of the barren soil, while none was found in the first foot of the soil where reasonably good growth of barley occurred. However, substantial amounts of sodium bicarbonate were found in these latter samples, and both sodium carbonate and sodium bicarbonate occurred in all but one of the corresponding samples of the second foot. It is quite possible that the growing barley gave off CO_2 in amounts sufficient to convert the normal carbonate of the first foot into the bicarbonate.

Each sample of series B contained very low concentrations of the neutral salts, but again, the barren portion contained rather high concentrations of sodium carbonate. It will be noted also that small amounts of sodium carbonate were found where a fair crop of barley was secured, but in this case the second foot, with the single exception of sample number 8, contained considerably less normal carbonate than the first foot.

TABLE 8

Composition of soil samples, series B, second foot

SAMPLE NUMBER	1	2	3	4	5	6	7	8
	Parts per million							
CO_3	60	105	120	60	15	0	15	45
HCO_3	274	198	244	274	305	274	274	290
Cl	115	97	89	106	160	115	62	80
SO_4	41	41	47	41	82	31	17	Trace
NO_3	0	0	0	3	5	0	5	0
Total salts	800	800	820	760	920	650	390	575
pH value	9.2	9.2	9.5	8.8	8.8	8.0	8.8	9.2

The samples composing series C were taken at a uniform distance of two feet apart. The area sampled was entirely devoid of vegetation. Barley seeds had failed to germinate on this spot for several years previously. The analysis of the first, second, third and fourth feet, respectively, are submitted in tables 9, 10, 11 and 12.

It will be noted that the chloride, sulphate, nitrate and total salts varied greatly. Among the samples of the first foot, number 8, for example, contained more than five times as much chloride, sulphate and nitrate as sample 9 taken just two feet away. Other samples (4 and 6) taken 4 feet apart, varied more than four-fold while in one instance samples taken 6 feet apart (6 and 9) showed more than a ten-fold difference in their neutral salts. On the other hand, the content of alkali carbonate was comparatively constant throughout this series.

Considerable variation was also noted among the samples from the second, third and fourth feet. Two of the samples (9 and 10) contained greater amounts of chloride and sulphate in the second than in the first foot, while the reverse is true of the other samples.

TABLE 9

Composition of soil samples, series C, first foot[*]

SAMPLE NUMBER	1	2	3	4	5	6	7	8	9	10
	Parts per million									
CO_3	465	390	360	420	360	525	450	285	420	450
HCO_3	305	320	457	305	457	335	214	640	214	335
Cl	2080	1968	1914	656	1489	2659	1452	1179	177	674
SO_4	1844	1148	1030	433	1098	2012	735	575	55	506
NO_3	400	250	249	89	312	481	267	223	43	116
Total salts	7740	7070	6555	3085	5955	10250	5130	4900	2405	3770
pH value	9.6	9.6	9.6	9.6	9.7	9.8	9.8	9.3	9.6	9.8

[*] Samples were taken 2 feet apart.

TABLE 10

Composition of soil samples, series C, second foot

SAMPLE NUMBER	1	2	3	4	5	6	7	8	9	10
	Parts per million									
CO_3	300	360	360	360	375	300	270	240	330	300
HCO_3	274	244	259	198	244	305	366	290	214	290
Cl	833	638	381	567	780	1072	372	523	1028	1117
SO_4	608	218	95	207	340	410	76	128	709	655
NO_3	142	85	46	62	134	151	53	89	169	116
Total salts	3110	2570	1820	2375	2515	3555	1850	2325	4170	4200
pH value	9.6	9.6	9.6	9.6	9.7	9.8	9.7	9.3	9.6	9.6

TABLE 11

Composition of soil samples, series C, third foot

SAMPLE NUMBER	1	2	3	4	5	6	7	8	9	10
	Parts per million									
CO_3	315	330	300	300	270	240	240	240	210	315
HCO_3	335	274	214	366	305	305	351	320	229	259
Cl	355	266	381	230	195	355	186	275	239	301
SO_4	107	100	84	31	41	124	11	46	41	42
NO_3	53	29	37	23	20	50	15	32	29	22
Total salts	1850	1650	1675	1365	1280	1695	1220	1075	1435	1650
pH value	9.6	9.6	9.3	9.7	9.2	9.6	9.4	9.5	9.2	9.6

TABLE 12

Composition of soil samples, series C, fourth foot

SAMPLE NUMBER	1	2	3	4	5	6	7	8	9	10
	Parts per million									
CO_3	180	150	150	165	60	60	90	75	90	330
HCO_3	320	335	305	274	305	351	214	351	259	168
Cl	257	319	142	97	142	346	177	133	115	230
SO_4	83	98	58	21	47	181	43	34	31	62
NO_3	24	20	12	9	12	26	18	15	11	18
Total salts	1320	1400	920	930	675	1280	835	830	900	1485
pH value	9.4	9.5	9.0	8.9	8.7	8.9	8.9	9.0	8.9	9.7

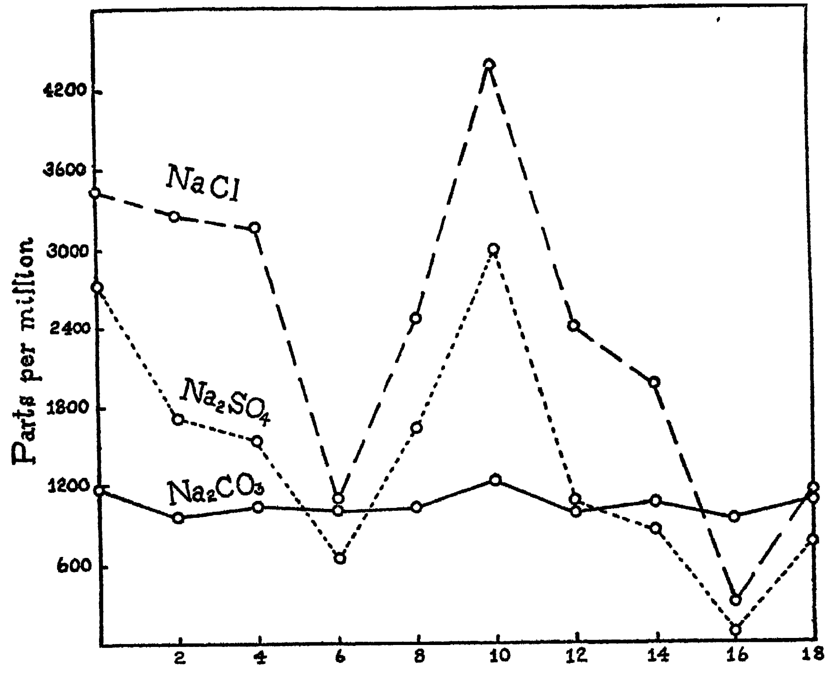


FIG. 3. GRAPHS SHOWING THE LINEAR VARIATION OF AN ALKALI SOIL AT DISTANCES OF TWO FEET APART, FIRST FOOT, SERIES C

Na_2CO_3 calculated from methyl orange titrations

DISCUSSION

The preceding data show that the amount of some one or more of the soluble salts in soil samples taken from a very small area varied enormously. The distribution of chloride and sulphate was especially variable. Samples taken only 2 feet apart showed a difference of more than 600 per cent in chloride and more than 1000 per cent in sulphate content. As is illustrated by the graphs, the concentration of the several neutral salts was much more variable than that of the alkali carbonates, although the distribution of the latter was far from being uniform.

It is evident from these data that the analysis of a single soil sample drawn from one place within the area studied, has very little value. It was found, for example, that one or more samples from each of several of the experimental plots contained practically no alkali salts; other samples contained high concentrations of one or more salts; and still others had a composition intermediate between these extremes. If similar variation characterizes the distribution of salts in alkali soils generally, it may be safely concluded that the analysis of samples such as are commonly submitted by practical farmers is a waste of time. In fact, the conclusions that are likely to be drawn from the analysis of such samples may be so erroneous as to lead to the recommendation of practices the very opposite of those that should be employed.

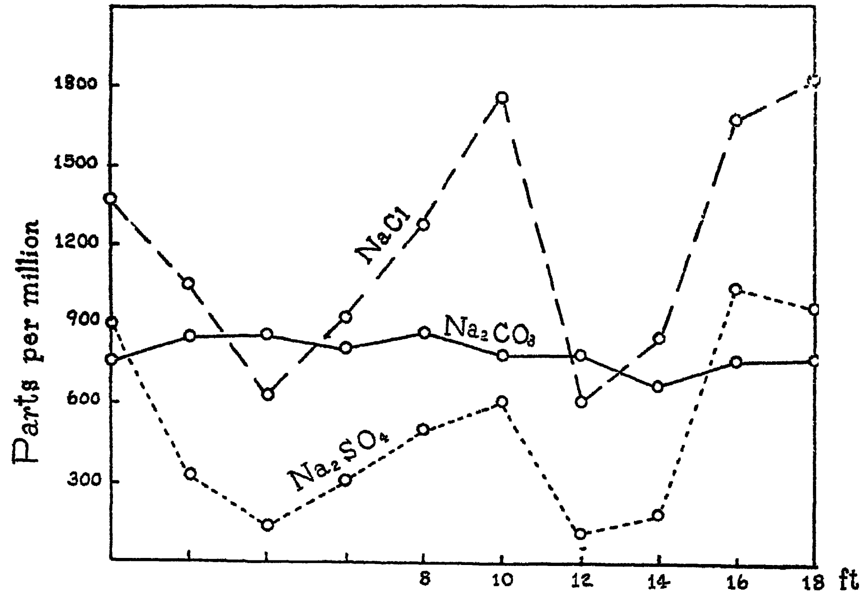


FIG. 4. GRAPHS SHOWING THE LINEAR VARIATION OF AN ALKALI SOIL AT DISTANCES OF TWO FEET APART, SECOND FOOT, SERIES C

Na_2CO_3 calculated from methyl orange titrations

The character of the vegetation probably affords one of the safest guides in the sampling of an alkali soil. Wherever noticeable differences occur in the vigor of a given species or in the distribution of different species, there is likely to be some difference in the salt content of the soil. Poor growth of a crop, however, does not necessarily denote alkali soil, for any or all of the factors which limit plant growth in humid regions, many of which are very imperfectly understood, may be present in the soils of alkali regions.

It seems quite doubtful whether a composite sample of an alkali soil can be relied upon unless it be composed of a large number of individual samples each of which was taken from an area in which the variation lies within reasonable limits. At present it is not possible to say what number of individual samples will be required to determine the mean composition of a given area of alkali soil. If the area be of considerable size the number of samples is reasonably certain to be very large.

In a subsequent paper the reclamation of this soil will be discussed. It is of interest to point out at this time that the application of a given treatment has already proved highly successful with certain parts of these plots and quite unsuccessful on other parts. In undertaking plot experiments on this or other similar soil, it is scarcely possible to over-emphasize the importance of the extreme variability of the soil.

The above results suggest some of the reasons why it is often found difficult to determine the alkali tolerance of plants. In the first place, alkali soils usually contain more than one kind of salt. In the second place, it is rare that the concentration of one salt varies while that of the others remains constant. Thirdly, the soil on one side of a plant may be highly charged with salts and on the other side comparatively free from salts. In view of these facts it is doubtful whether an accurate determination of alkali tolerance can be made by means of field cultures alone.

REFERENCES

(1) Kelley, W. P., and Brown, S. M. 1921 The solubility of anions in alkali soils. *In* Soil Science, v. 12, p. 261–285.

(2) Linhart, George A. 1920 A new and simplified method for the statistical interpretation of biometrical data. *In* Univ. Cal. Pub. in Agr. Sci., v. 4, no. 7, p. 159–181.

(3) McBeth, I. G. 1917 Relation of the transformation and distribution of soil nitrogen to the nutrition of citrus plants. *In* Jour. Agr. Res., v. 9, no. 7, p. 183–252.

(4) Scofield, Carl S., and Headley, Frank B. 1921. Quality of irrigation water in relation to land reclamation. *In* Jour. Agr. Res., v. 21, no. 4, p. 265–278.

(5) Waynick, Dean David. 1918 Variability in soils and its significance to past and future soil investigations. I. A statistical study of nitrification in soils. *In* Univ. Cal. Pub. in Agr. Sci., v. 3, no. 9, p. 243–270.

(6) Waynick, D. D., and Sharp, L. T. 1919 Variability in soils and its significance to past and future soil investigations. II. Variations in nitrogen and carbon in field soils and their relation to the accuracy of field trials. *In* Univ. of Cal. Pub. in Agr. Sci., v. 4, no. 5, p. 121–139.

THE SOIL SOLUTION, EXTRACTED BY LIPMAN'S DIRECT-PRESSURE METHOD, COMPARED WITH 1:5 WATER EXTRACTS

PAUL S. BURGESS[1]

Rhode Island Agricultural Experiment Station

Received for publication November 21, 1921

INTRODUCTION

The physical, chemical and biological complexity of arable soils is everywhere recognized. Soils may be considered as being made up of three distinct classes of physical components or phases; the solid, the liquid and the gaseous. Until recent years the solid phase has received the lion's share of attention, but we are beginning to realize the importance of more definite knowledge concerning the gaseous and especially the liquid portion of soils. This is necessary if we are to understand more perfectly soil interrelationships and physiological plant processes. The analysis of soil gases has from early times been a subject of interest and somewhat extended study, especially the oxygen: carbon-dioxide ratio.[2] The liquid phase, however, as it exists in the interstices and as it intimately surrounds the soil particles at approximately optimum moisture content, although receiving much opinionated theoretical discussion, has to its credit a surprising lack of experimental data. This, of course, is due to the fact that numerous difficulties are at once encountered when one attempts to separate the natural soil solution from its harboring solid phase.[3] That a furtherance of our knowledge of this, the universal plant nutrient solution, is indispensable to the advancement of our science, is now admitted by most students of soils. At one time it was contended that all normal, tillable soils contain solutions of approximately equal concentrations, and that work on solutions from different soils was but a repetition of effort. This erroneous theory has fortunately been refuted and the value of such studies is now widely recognized Not only is it desirable to ascertain the ratios, and absolute concentrations of the so-called mineral nutrient elements dissolved in soils of different productive capacities, but also is a knowledge of the concentrations of certain inorganic toxic substances, such as aluminum, manganese, ferrous iron, hydrogen ions, and the so-called "alkali salts," in the soil solution[4] of decided importance. Exact quantitative knowledge regarding the natural liquid phase of soils may further enable us to evaluate more accurately the data obtained by means of water extracts. Definite knowledge concerning the natural soil solution is necessary to an understanding of soil-fertility problems. And finally, those minute and indispensable allies to continuous productivity, the soil microörganisms, live, function in and derive their food supply from this same imperfectly known soil solution.

---

[1] The work herein reported was performed in the laboratories of the University of California. It is a pleasure to the writer sincerely to thank Dr. C. B. Lipman, Dr. W. P. Kelley and Prof. D. R. Hoagland for their many helpful suggestions and timely criticisms.

[2] The more recent and exacting work of Russell and Appleyard in England, Lyon and Bizzell in America, and Harrison, et al., in India has greatly extended our knowledge along this line.

[3] Quincke's figures show that solid particles the size of "clay" are able to hold thin water films to their surfaces with a force equivalent to approximately 300,000 pounds to the square inch, while Lord Rayleigh, from certain experimental data, calculated that thin films of liquids (water among them) were held to certain surfaces with a force of 25,000 atmospheres per square inch.

The present paper presents a quantitative chemical study of the liquid phase of soils as extracted by a direct pressure method. The idea of using heavy pressures, as secured on large testing machines, for this purpose was proposed by Lipman (10) in 1918 after a few preliminary experiments had shown its feasibility. The procedure was at the time quite severely and somewhat caustically criticized by Northup (15). That this criticism was founded largely upon opinion, and can hardly be said to have been substantiated by fact, will be shown by subsequent data.

PREVIOUS WORK

It is not the purpose of the writer to review all of the past attempts at securing the soil solution as it exists naturally in soils. This has been quite fully done by Morgan (14) on two separate occasions. He groups such procedures under the general headings of drainage waters, soil extracts, artificial roots, centrifuge and displacement methods. No mention was made by him of direct-pressure or "squeezing" methods, although a few such attempts have been recorded.

Probably De Saussure (19) was among the first to secure a portion of the soil solution for analytical purposes. Direct pressure applied to the moist soil was used by him. He filled large vessels with soils containing considerable quantities of humus. These were then saturated with pure water and allowed to drain for a period of five days. The soils were then subjected to powerful pressures and the water thus extracted was analyzed quantitatively for carbon dioxide and tested qualitatively for other substances.

More recently van Zyl (23), experimenting largely with clay soils, employed direct pressure. He found no difficulty in securing considerable quantities of soil solution by this means. Analyses of such solutions from different soils showed decided differences in the amounts of plant food constituents present. He stated that this was due to previous fertilization and to climatic factors.

---

[4] The reader should not confuse the terms "soil solution" and "soil extract." The former is the soil water as it exists naturally in normal field soils, while the latter is a solution secured by extracting a soil with various solvents, usually used in excess of soil weight, and under decidedly artificial conditions. The several solvents employed in such extractions may yield solutions far removed in quantitative composition from the true soil solution.

Studies later conducted at the University of Göttingen by Ehrenberg and van Zyl (6) on soil solutions are also of interest. Loam and clay soils were subjected to high pressures, using heavy hydraulic presses of great capacity. Comparisons of solutions thus obtained from soils differently fertilized are reported. Unfortunately the pressures used were not stated.

Ramann, März and Bauer (18), after having shown drainage waters to reflect but poorly the composition of true soil solutions, also adopted the method of forcing the capillary water out of soils as taken from the field, with a hydraulic press. They employed a pressure of 300 kgm. per sq. cm. (4300 lbs. per sq. in.). The expelled liquids were analyzed for calcium, magnesium, potassium, phosphoric acid, and sulfates. The soil samples were taken once each month, beginning in May and ending with October. Both surface soils and subsoils were used. The monthly variations of the different ions under growing crops were discussed, together with the effects of climatic factors on the fluctuations noted. The solutions obtained from the subsoils were all of fairly constant composition and of low concentration.

A rather ingenious method of obtaining the soil solution was recently proposed by Taylor (22). He buried pads of absorbent filter paper in moist soils for definite lengths of time and then extracted the absorbed solutions from the pads by subjecting them to high pressures. The solutions thus procured were subsequently analyzed as were the soils themselves (acid extraction). By a comparison of such analyses it was concluded that "the composition of the soil water is not that which would be inferred from the chemical analysis of the corresponding soil."

An interesting paper, reviving the old alcohol-displacement method proposed nearly fifteen years ago by Ischerekov (9), has recently appeared by Parker (16) in which yields of 36 to 75 per cent of the total soil solution were obtained. A few preliminary experiments by the writer showed displacements approaching 60 per cent for fine sandy and silt loams, but for the heavier soils, several days were often required in securing a small fraction of the soil solutions present at optimum moisture contents. The method, however, has promise and should receive the careful consideration of soil chemists.

Before taking up a discussion of the direct pressure method for extracting the soil solution, I desire very briefly to record certain experiences with the oil-pressure procedure as suggested by Morgan (14), which were secured at the expense of the Agricultural Experiment Station of the Hawaiian Sugar Planters' Association in Honolulu in 1917 and 1918. Three widely different types of Hawaiian soils were used: clay loams, sandy loams and a highly organic rice soil. Various mechanical devices and different methods of packing the soils were tried. Two grades of paraffin oil (light and medium heavy) were used, and pressures up to 1000 lbs. per square inch were employed. The inside diameter of the cylinder used was 10 inches and its height was 18 inches.

It was found first of all that the oil did not penetrate the soils, forcing the moisture out ahead of it, as was claimed. One inch of penetration was the maximum ever noticed, even in the sandy soils and under pressures up to 1000 pounds per square inch, all due precautions being taken. This was the case irrespective of grade of oil used. The oil under pressure acted as a solid ram or piston. The clay and organic rice soils were very springy, contracting and expanding like a sponge as the pressure was turned on and off, but practically no penetration of the oil into the soil mass was noted. The moisture which was expressed came almost entirely from the lower layers of the soils, as the following figures taken at random from many such data will show:

After pressing 6 hours at approximately 900 pounds,

	per cent
Original (soil No. 1)	47.0
Surface 4 inches (next to oil)	47.0
Second 4 inches	46.1
Third 4 inches	45.0
Last 3 inches (next to screens)	42.0

(Samples taken from cylinder with auger)

It was also found that but a very small fraction of the total moisture (when the soils are at optimum) can be secured by hydraulic pressures within reason; less than 20 per cent for the sandy soils, less than 2 per cent for the clay loams and less than 10 per cent for the peaty rice soil. In fact, only a few cubic centimeters of solution were secured from 30 to 40 pounds of several of the heavier clay soils pressed at 500 to 800 pounds presssure for several hours.

Finally, as stated by Lipman (10), the method is extremely "time-consuming and untidy." This criticism might well be waived if desirable results had been secured but this can hardly be said to have been the case. It should be borne in mind, however, that the writer here employed Hawaiian soils which differed greatly (both physically and chemically) from the arable soils commonly found on the mainland.

THE DIRECT PRESSURE METHOD

The objects of the experiments about to be described were: (1) To separate from the solid soil mass the true soil solution as it exists in soils when they are under approximately optimum moisture conditions for the growth of crop plants; (2) To ascertain the actual concentrations within these solutions of ions essential as food materials for plants; (3) To compare quantitatively these results with data from the same soils obtained by the water-extraction method.

Apparatus employed

As the name of the method suggests, heavy pressures were applied directly to moist soils held in properly constructed retaining vessels or presses which were provided with suitable exits for the escape of the expelled solutions. The design and construction of the retainers capable of withstanding pressures up to 100,000 pounds to the square inch, which would allow moisture to escape but at the same time hold back even the smallest particles of soils, was a task which consumed by far the larger portion of the total time spent by the writer upon this work, and he wishes here to express to Professor C. T. Wiskocil, in charge of the Testing Laboratories of the Department of Civil Engineering of the University of California, his most sincere thanks for the many helpful suggestions rendered and for the actual design of the last and most efficient press built for this work.

Three different presses were constructed in the machine shops of the Testing Laboratories during the course of the experiments. Press no. 1 was simply an 8-inch cylindrical steel block carrying a 2-inch hole through its center which was carefully finished to receive two movable, hardened and ground steel pistons. The interior was slightly recessed. A finely perforated brass sleeve of the same inner diameter as the pistons was placed in the recess, and thoroughly washed white sand packed between it and the inner wall of the retaining steel block. Through the block an opening for the expressed solution was provided. The soil was packed into the brass sleeve, the pistons inserted, one from the bottom and the other from the top, and the whole placed on the table of a heavy testing machine which supplied the pressure. As will be seen, the washed sand here acted as a filter or soil retainer. This type of press was most efficient in the separation of solutions from heavy clay and clay-loam soils although it was extremely time-consuming in operation.

The second press constructed, Press no. 2 (see plate 1), was the one used for obtaining a majority of the solutions later to be discussed. This one consisted of a heavy steel retaining block bored out on a slight taper to receive a sectional steel sleeve through which a 2-inch hole was carefully bored and ground to receive the hardened and ground pistons. Many rows of very small holes (one-ten-thousandth of an inch in diameter) were drilled from channels cut lengthwise along the backs of the four pieces of the sectional sleeve into the inner 2-inch finished hole. Through these fine holes the expressed soil solution passed. The taper and collapsible sleeve were made to facilitate the ejection of the hard briquettes of soil after pressing. This press could be efficiently used only with sandy soils, as the finer silts and clay soils were readily squeezed through the small holes. However, with all kinds of sandy soils, even with very fine sandy loams, the apparatus was a decided success, and rapid of operation.

Press no. 3 was without doubt the best one constructed, but it became necessary for the writer to leave California after having worked with it only a short time. It became evident while working with Press no. 2 that the small holes, which were supposed to allow only the solution to pass through, were too large to hold back the smallest soil particles. It was impossible, however, to drill smaller holes, hence some other device was necessary. The making of fine slits, rather than holes, suggested itself. The third press was constructed on this principle, and is shown in plate 2. The moist soil was packed into the center hole, as in the other presses described, and the pistons inserted and forced together against the soil by one of the testing machines. With all presses, considerable trouble was at first experienced because the finer textured soils crept back past the pistons in thin ribbons when the pressure was first applied. However a thin layer of washed sand next to the piston ends permanently overcame this difficulty.

The machines which supplied the pressure were of standard heavy types as found in laboratories where building materials are tested. Three machines were used at different times, one capable of giving 50,000 pounds total pressure per square inch, one of 100,000 pounds capacity (see plate 2), and one of 500,000 pounds capacity. The first two were motor-driven; the last, of hydraulic ram type. The smallest machine was usually employed since no moisture was expressed from the fine sandy loam soils at pressures above 16,000 pounds per square inch.

The soils employed

Much work has been reported during the past few years by Burd (2), Hoagland (7) and Stewart (21) of the California Agricultural Experiment Station on the effects of plant growth and season upon the concentrations of the soil solution as periodically manifested by water extracts of cropped and fallowed soils. Since an important feature of this present investigation was to compare water extracts with actual soil solutions, it was thought advisable to use the same soils for this work as had been employed previously by the above-named investigators. Through their courtesy sufficient quantities of certain of these soils were placed at the writer's disposal. They had been collected in 1914 and 1915, and since that time had been stored in water-tight bins in an air-dried condition. The numbers originally given to these soils have been retained throughout the present work. A brief description of the samples follows. All are California soils and have been mapped and named by the Bureau of Soils, United States Department of Agriculture.

Soil no. 7. Hanford fine sandy loam. From Arlington, southern California. Oats on land when sampled. Past treatment: originally grain; about 1890 put into alfalfa for 13 years; potatoes 2 years; alfalfa 4 years; oats 5 years. Yield of oats: 4 tons of hay per acre.

Soil no. 8. Fresno fine sandy loam. From Fresno, San Joaquin Valley. Seedless grapes on land when sampled. Past treatment: originally grain; 14 years in Sultanina grapes. Production: about 2 tons of raisins per acre for the last 6 years.

Soil no. 9. Kimbal fine sandy loam. From Redlands. Navel oranges on land when sampled; 25 years in oranges; formerly desert land. Heavily fertilized.

Soil no. 10. Tejunga fine sandy loam. From San Fernando Valley, southern California. Peaches on land when sampled; originally 15 years in prunes; last 10 years in peaches. Some stable manure used.

Soil no. 11. Madera fine sandy loam. From Kearney Park, San Joaquin Valley. Navel oranges on land when sampled; in oranges for past 15 years; formerly in grain. Well fertilized.

Soil no. 12. Yolo sandy loam. From the Unversity Farm at Davis, Sacramento Valley. This soil has been cropped to grain (wheat or barley) since about 1860.

Soil no. 14. Standish fine sandy loam. From the Honey Lake region, northern California. Virgin, desert soil; small shrubs and weeds, natural growth.

Soil no. 3. Imperial Valley silt. This sample was not secured from Professor Burd, but was collected by the writer in 1915 from near Holtville in the center of the Imperial Valley, southern California. It is a virgin, desert soil supporting a sparse growth of grease-wood (*Sarcobatus vermiculatus*) and creosote bushes (*Covillea Mexicana*). Annual rainfall, 2 to 4 inches.

Table 1 (21, p. 321) gives the moisture equivalents (method of Briggs and Shantz), hygroscopic coefficients (method of Hilgard), and the mechanical analyses (method of Bureau of Soils) of the soils used. From these data a fair idea of the physical properties of the several soils may be formed.

TABLE 1

Physical characteristics of soils employed

SOIL NUMBER	MOISTURE EQUIVALENTS	HYGROSCOPIC COEFFICIENTS	MECHANICAL ANALYSES				
			Coarse and medium sand	Fine sand	Very fine sand	Silt	Clay
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
7	13.31	2.89	5.69	15.60	53.67	16.01	9.77
8	8.06	1.17	5.14	21.22	50.63	17.57	5.56
9	9.87	1.53	16.30	21.73	43.67	10 87	8.19
10	18.38	2.94	4.51	16.62	38.76	26 25	13.37
11	16.28	3.16	5.59	20.95	39.34	19.08	14.62
12	9.72	1.77	9.37	23.27	46.24	9.61	9.44
14	17.99	4.74	7.14	24.54	38.94	15.55	13.87

Preliminary experiments

In order to establish certain important facts as well as to answer certain criticisms (15) of the pressure method, a number of preliminary experiments were first performed. It was deemed necessary to ascertain the effect of the different pressures upon total soil solubility, upon the specific gravity of the soil solutions extracted, upon their specific conductivities and upon the solubilities of the more important elements. Furthermore, it was thought desirable to know whether such pressures would effect a measurable fining or abrasion of the soil particles, thereby exposing fresh surfaces to the solvent action of the soil moisture during extraction. We realized that bacteria are killed at high pressures, hence no work of a bacteriological nature was attempted on any of the solutions obtained.

An increase in pressure has been supposed to enhance the solvent action of water on minerals and soils, but so far as the writer has been able to find, little direct proof of this fact is extant. That this supposition, in some cases at least, is erroneous has been shown by Spezia, by Tammann and also by Van Stackelberg (4, p. 31). While there is some geological evidence to show that the solvent action of water at great depths in the earth's crust is slightly greater than at the surface, Cameron and Bell (4) state that probably here the increases are largely due to higher temperatures and to the increased concentration of gases in solution, notably carbon dioxide. Some work has been done on the effect of direct pressures on the solubilities of salts dissolved in pure water, but here again the data is meager and often conflicting, as in the well-known case of ammonium chloride. It would seem probable that slight temperature fluctuations while working at high pressures might possibly account for very small differences occasionally noted, for while temperature change makes a vast difference in degree of solubility, exceedingly high pressures even upon concentrated solutions are required to effect the smallest measurable differences. In the light of such data as was available and in the light of information obtained by discussing the subject with chemists, it hardly seemed probable that great differences would be found in the concentrations of so dilute a solution as the natural soil water as a result of the pressures here employed.

In an endeavor to test this matter experimentally the expressed solutions from a number of soils were obtained in fractions depending upon the pressures used and determinations were made of the total soluble solids, soluble organic matter and dissolved inorganic matter on aliquots of these several fractions. The soils were kept under approximately optimum moisture conditions for two or three days immediately before pressing. Those here used were all sandy loams of varying degrees of fineness. The three fractions were so regulated as to give approximately equal volumes of solution. The results appear in table 2.

As will be seen by studying these data, the quantity of materials dissolved in the different fractions of the soil water extracted from a given soil under different pressures varied but slightly in amount. This holds for the organic as well as for the inorganic solids It is thus highly probable that the heavier pressures used had but slight effect, if any, on the amounts of materials which were dissolved in the solutions of the three soils prior to pressing. *We cannot, of course, be sure that the higher pressures, or the lower ones for that matter, were actually applied to the liquid phase itself, as this was always free to escape.* It would seem somewhat more probable that most of the force was utilized in overcoming the friction of the solid phase caused by its compression into a slightly smaller space. From the above data it would also appear that the solutions remaining in these soils after applying the pressures were probably of similar concentration to those removed, and that still greater amounts of solution might have been obtained had the solid particles of the soil been of a more yielding or compressible nature.

The specific gravities of the three fractions of soil solution removed from soil 12 were determined by the picnometer method. The results were as follows:

	specific gravity
First fraction (0–1600 lbs.)	1.0010
Second fraction (1600–5730 lbs.)	1.0009
Third fraction (5730–15925 lbs.)	1.0010

As the results here secured are very nearly identical, and as determinations subsequently made practically proved the identity of the several fractions, this determination was not made on the fractions of the other soil solutions obtained.

TABLE 2

The effect of different pressures on soil solubilities

SOIL NUMBER	PRESSURE FRACTION	WATER EXTRACTED FROM EACH 400-GM. CHARGE OF MOIST SOIL	TOTAL SOLUBLE SOLIDS	INORGANIC SOLUBLE SOLIDS	ORGANIC SOLUBLE SOLIDS
	lbs. per sq. in.	*cc.*	*p.p.m.**	*p.p.m.*	*p.p.m.*
12	0– 1600	12–13	1340	890	450
12	1600– 5730	10–11.5	1310	870	440
12	5730–15925	7– 8	1350	860	490
10	0– 1600	15	2120	1000	1120
10	1600– 5730	14–15	2240	1090	1150
10	5730–15925	12–13	2280	1020	1260
7	0– 1600	14	1610	810	800
7	1600– 5730	15–16	1750	910	840
7	5730–15925	11–12	1760	920	840

* Parts per million of soil solution.

Specific resistance (the reciprocal of specific conductivity) determinations were made on all of the fractions secured. While this determination does not show the concentration of non-electrolytes and of un-ionized electrolytes, it was thought to be of importance as a sensitive indicator of total ionic concentration, and due to its dilution, the mineral or salt content of the soil water was thought to exist very largely in the ionic state. Table 3 presents these data.

The specific resistances of the several pressure fractions from each of the three soils are within the limits of experimental error, denoting almost identical ionic concentrations in each case.

As a final test on the chemical similarity of the solutions secured at different pressures, calcium and magnesium determinations were made on the several fractions. These two elements were chosen as here best reflecting ionic concentrations in general for they were known to be present in largest amounts, would thus be able to be determined with greatest accuracy in the small amounts of solution available, and would finally be capable of best showing small fluctuations in concentration. These data appear in table 4.

These data, taken in conjunction with those previously presented, would seem to prove that, with fine sandy loam soils at least, the application of direct pressures up to approximately 16,000 pounds per square inch has little, if any, effect on the quantitative composition of the expressed solutions, and suggest that the concentration of that part of the soil water which remained in the soil after pressing probably carried similar amounts of solutes at the time of extraction.

TABLE 3

Specific conductivities of soil solutions extracted at different pressures

SOIL NUMBER	PRESSURE FRACTION	SPECIFIC RESISTANCE
	lbs. per sq. in.	*ohms*
12	0- 1600	614.4
12	1600- 5730	624.6
12	5730-15925	627.2
10	0- 1600	354.6
10	1600- 5730	364.8
10	5730-15925	369.3
7	0- 1600	448.0
7	1600- 5730	450.6
7	5730-15925	444.8

TABLE 4

Calcium and magnesium determinations on soil solutions extracted at different pressures

SOIL NUMBER	PRESSURE FRACTION	CALCIUM	MAGNESIUM
	lbs. per sq. in.	*p.p.m.*	*p.p.m.*
12	0- 1600	150	87.5
12	1600- 5730	152	89.0
12	5730-15925	150	88.0
10	0- 1600	147	30.5
10	1600- 5730	165	37.1
10	5730-15925	165	36.0

The last test of the method was made in an endeavor to determine whether or not there was a grinding or fining of the soil when pressures of 16,000 pounds per square inch or less were applied. Such action, if it took place, might have a tendency to alter slightly the chemical composition of the resulting solution by exposing the fresh cleavage surfaces of the mineral particles to the dissolving effect of the liquid phase. McCool and Millar (12), working with the cryoscopic method, have, in fact, recently shown that grinding soils increases their solubility in distilled water, marked changes being observed in less than 24 hours. Suspension or subsidence tests were accordingly made on the moist soils, both before and after pressing, as follows: The equivalent of 10 gm. of water-free soil was triturated in a little distilled water with a rubber-tipped pestle to break up all lumps. These suspensions were then made up to exactly 250 cc. and shaken for 1 hour in a shaking machine when they were immediately poured into tall, glass-stoppered, graduated cylinders of the same bore and height, and allowed to settle for varying lengths of time, depending upon texture. Exactly 50 cc. of the turbid suspensions were then carefully removed from the surface of each cylinder, evaporated to dryness and the solid matter gravimetrically determined. In several cases the coarser particles which settled to the bottom were examined under a microscope in an endeavor to locate freshly chipped or abraded surfaces. The data obtained from the suspension experiments appear in table 5.

As will be seen, the solid matter in the several subsidence fractions which were at the top and obviously carried the smallest particles of the soils, was not greatly altered in amount by the pressure treatment. While it is possible that some of the larger particles may have been broken, we are here more especially concerned with the finer portions, for Loughridge (11), and more recently McCool and Millar (12) have shown that practically all of the materials which readily go into solution in soils come from the silt and clay fractions. No definite abrasive action was noted, however, in the microscopical examinations of the larger mineral particles. A complete mechanical analysis might possibly have settled this point better but lack of time prevented such extended preliminary work.

TABLE 5

Suspension tests to note the effect of pressure on abrasion of soil particles

SOIL NUMBER	PERIOD OF SUBSIDENCE	SUSPENDED MATTER BEFORE PRESSING	SUSPENDED MATTER AFTER PRESSING
	hours	*p.p.m.**	*p.p.m.**
12	4.5	1390	1354
10	2.5	2050	1820
7	1.5	1530	1600
8	8.0	570	740
9	4.5	830	1030
11	5.0	1290	1130

* Parts per million of suspension.

Percentages of soil solution extracted

It was the practice of the writer to ascertain first the soil's optimum moisture content,[5] bring it up to this percentage with distilled water, and allow it to stand, loosely covered, at room temperature for about a week (moistening when necessary) before the extraction was made. The soil was thoroughly stirred immediately before pressing and moisture determined. After pressing, another moisture determination was at once made. The difference between these gave the actual percentage of moisture expressed, calculated as percentage of the dry soil.

---

[5] Fifty per cent of saturation as shown by Hilgard's method.

Table 6 records the percentages of moisture in each soil before and after pressing, the actual percentages of soil water extracted, their wilting coefficients as calculated from the mechanical analyses by the formula proposed by Briggs and Shantz, and the percentages of "unfree water" as calculated for these soils by Hoagland (7) from freezing point determinations.

A perusal of table 6 reveals certain points of interest. The first is that, of the eight fine sandy loam soils moistened to but 50 per cent of their total moisture-holding capacities, over 60 per cent of this water was extracted in five cases, over 53 per cent in two cases and about 45 per cent in the final instance. Not more than 16,000 pounds pressure per square inch was ever required, and it should be stated that very little solution (none in most cases) was secured above 10,000 pounds pressure per square inch, although, to be sure of uniform treatment, the final pressure used was in all cases 16,000 pounds.[6] In practically every instance, at least three-quarters of the soil solution was obtained before the gauge registered 20,000 pounds total pressure (6,370 pounds per square inch of piston surface). Another interesting fact is that, in almost all cases, the percentage of moisture in the soil after pressing was within less than one per cent of its calculated wilting coefficient. In other words, the amount of soil water removed by direct pressure was approximately the same as that which plants are able to secure when growing under proper conditions.

TABLE 6

Percentages of soil moisture extracted

SOIL NUMBER	MOISTURE IN SOIL BEFORE PRESSING*	MOISTURE IN SOIL AFTER PRESSING*	MOISTURE EXTRACTED FROM SOIL	WILTING COEFFICIENT (CALCULATED)	"UNFREE WATER" (7, P. 371)
	per cent	*per cent*	*per cent of total*	*per cent*	*per cent*
3	22.7	9.0	60.3		
7	19.1	6.6	65.4	7.2	6.0
8	18.3	8.7	52.5	4.5‡	4.0
9	17.7	9.9	44.8	10.8	6.0
10	23.0	8.4	63.5	10.1	8.0
11	17.8	6.9	61.2	8.9	6.0
12	21.9†	8.0†	63.4	6.6	4.0
14	22.0	10.4	52.7	9.8	7.5

* Figured on moisture-free basis.

† Moisture data on fractional extractions only were secured.

‡ This wilting coefficient is apparently too low.

---

[6] Pressures up to 100,000 pounds were employed in a few cases, after the usual pressing but, from the fine sandy loam soils, no more solution was ever forced out. This was *not* the case with the other soil types. Very little moisture was expressed from clay loams until at least 15,000 pounds per square inch had been applied.

The so-called "unfree" or "combined" water of soils as determined by Bouyoucos (1) (that fraction which cannot be frozen) has been determined for these soils by Hoagland (7, p. 371), and is given for reference in the last column of the table. By comparison with data shown in column 3, we see that in some cases, the moisture content has been reduced by pressing almost to the point of "unfree water," while the average difference for all determinations is but a little over 3 per cent above the mean "unfree water" content.

The analyses of the soil solutions

About 500 cc. of soil solution were required for the several determinations made. As from 30 to 40 cc. of solution was obtained per single pressing (about 400 gm. of moist soil to the charge), from 15 to 20 pressings from each soil were usually necessary. With Press no. 2, each portion required about 30 minutes, so that an entire day's work was ordinarily necessary to secure the needed volume of liquid. The solutions were immediately taken to the laboratory, filtered through paper if turbid, separated into the aliquots required for the several determinations, and the evaporations started at once so as to avoid any possible bacterial alteration of the solutions. Calcium was determined volumetrically, and magnesium, gravimetrically, in 100 cc. aliquots, phosphates (21) in 200 cc. aliquots, potassium (21) in 100 cc. aliquots, while sulfates were gravimetrically determined on the residue from the inorganic solids portion. Nitrates were determined by the phenol-disulfonic-acid method on a 25 cc. aliquot. The conductivity measurements were made on the 200 cc. portions which were subsequently used for the phosphate determinations. Due to the usual presence of considerable amounts of nitrates, the hydrogen-ion concentration measurements were made colorimetrically on 8 or 10 cc. of the solutions. The results of these analyses appear in table 7.

TABLE 7

The chemical composition of the soil solutions

SOIL NUMBER	PERCENTAGE OF SOIL WATER EXTRACTED	SPECIFIC RESISTANCE	REACTION	TOTAL SOLUBLE SOLIDS	INORGANIC SOLIDS	Ca	Mg	K	NO_3	PO_4	SO_4
	per cent	*ohms*	*pH*	*p.p m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p m.*	*p.p.m.*	*p p.m.*	*p.p m.*
3*	60.3	635.0	7.3						97		
7	65.4	454.4	6.9	1950	1110	270	62	42	389	2	173
8	52.5	457.6	7.0	2120	1210	223	65	67	549	2	251
9	44.8	480.6	7.0	1760	1010	223	43	33	212	1.5	177
10	63.5	377.6	7.1	3300	1500	355	97	81	487	2.5	170
11	61.2	211.2	6.9	4300	2490	452	154	108	761	5.5	293
12	63.4	600.0	7.0	1000	630	110	67	16	71	1.5	
14	52.7	475.1	6.9	1920	1090	208	55	82	566	1.8	148

* Not completely analyzed.

The concentrations of the several ions have all been figured to parts per million of soil solution. Considerable silica and sodium, as well as iron, were noticed in several of the soil solutions during the course of the analyses. These were not quantitatively determined, except the iron in the case of soil solution 10. This solution carried 14 p.p.m. of dissolved iron (Fe). Slight precipitates of ferric hydroxide were noticed upon adding ammonium hydroxide in most of the solutions, prior to the calcium precipitation.

The specific resistance measurements are interesting in that they show the total ionic concentration of the soil solutions secured. Recalling that resistance decreases with increased concentration, we see that, with the exception of soil 7, a correlation is in all cases possible between the total dissolved solids as tabulated in column 5 and the resistances in ohms as shown in column 3. The order of decreasing concentration is as follows: soils 11, 10, 7, 8, 14, 9 and 12. By referring to Burd's (3) discussion of the relative fertilities of these same soils as shown by cropping tests we find the following classification: Good, 11, 8 and 14; medium, 10 and 7; poor, 9 and 12.

Using the freezing-point method of Bouyoucos, Hoagland (7) has calculated the concentration of total dissolved solids in the soil solutions of these soils under approximately optimum moisture conditions. The following table presents the averages of his results as secured periodically over a period of several months from the soils of the tank experiment, together with those directly determined on the soil solutions of the same soils by the writer.[7]

TABLE 8

Calculated and determined concentrations (dissolved solids) of soil solutions

SOIL NUMBER	CALCULATED BY HOAGLAND FROM LOWERED FREEZING POINT		DIRECTLY DETERMINED ON BIN SOILS
	Fallowed	Cropped	
	p.p.m.	*p.p.m.*	*p.p.m.*
7	3666	1830	1950
8	3850	1700	2120
9	2250	1400	1760
10	3200	1750	3300
11	4200	1800	4300
14	3000	2100	1920

The results determined directly from the expressed solutions are in most cases much lower than those calculated from freezing point data on the fallowed soils but higher than those from the cropped soil. There are points of similarity, however. For instance, both methods show soil 11 to carry the solution of highest concentration while soil 9 gives the lowest results. It should be recalled in making these comparisons that, while originally, each fallowed, cropped and bin soil was identical. differences in subsequent treatment have altered their amounts of readily soluble materials.

---

[7] While the samples for all of this series of determinations came from the same original lots of soil, those experimented upon by Hoagland had been subsequently cropped and otherwise differently treated from those used by the writer, which had been stored in bins for the past five years in an air-dried condition.

Column 4 of table 7 shows all of the soil solutions to be practically neutral in reaction. The hydrogen-ion concentration determinations were made by the colorimetric method.

The concentration of calcium ions is consistently high when compared with similar data for the more humid eastern soils as given by Morgan (14), using the oil-pressure method. The range found by the writer varies from 200 to 300 p p.m. of soil solution, whereas for Michigan soils 100 to 200 p.p.m. is the rule. Concentration of magnesium ions in the soil solutions from the California soils studied, range from 60 to 100 p.p.m of solution, while slightly higher results are reported by Morgan for humid soils. Potassium-ion concentrations are from two to three times as great in the solutions from western soils; while, as might be expected, the nitrate ion is a variable factor in both cases. The concentration of the phosphate ion is exceedingly low and shows but slight variations, either as between eastern and western soils or between the solutions from soil of different degrees of productivity (1, p. 305). The total range was from 1.5 to 3.5 p.p.m. of solution. Sulfates varied from about 150 to 300 p.p.m.

If the reacting values of the ions[8] are calculated, and the total weights of basic and acidic ions compared, there will be found to be a great preponderance of basic ions in all of the solutions reported. This is doubtless due to the fact that the HCO_3 ions as well as the Cl and SiO_3 ions were not determined and hence could not be used in these computations. That considerable quantities of calcium carbonate and bi-carbonate were present in these soils was shown by Stewart, and tests by the writer showed slight effervescence with HCl in one or two cases.

Water-extraction studies

Since an important part of this work was to be a quantitative comparison between the soil solution extracted by the direct pressure method and 1:5 water extracts of the same soils, portions were extracted with CO_2-free water and the resulting solutions analyzed for the Ca, Mg, K, NO_3, PO_4 and SO_4 ions.

The extracts were made by placing the equivalent of 400 gm. of dry soil in a clean, dry, acid bottle, adding sufficient water to effect the ratio of 1 part of soil to 5 of solvent, and shaking in an end-over-end shaking machine (10 r.p.m.) for exactly 1 hour. The solutions were allowed to settle and then filtered through Pasteur-Chamberland porcelain pressure filters rejecting the first 200 cc. On these perfectly clear solutions the determinations were made. The methods used were similar to those already described for the soil solution work, except that larger aliquots (400 to 600 cc.) were here found necessary.

---

[8] The valence divided by the atomic weight, or the ionic weight as the case may be.

Specific resistance determinations were also made on these extracts. Hydrogen-ion concentrations were determined on the soils themselves by the electrometric method.

The results secured are recorded in table 9, as parts per million of the soil extracts. To convert this data into parts per million of water-free soil, multiply by five.

TABLE 9

The chemical composition of the 1:5 water extracts

SOIL NUMBER	SPECIFIC RESISTANCE	REACTION	Ca	Mg	K	NO_3	PO_4	SO_4
	ohms	*pH*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p m.*	*p.p.m.*	*p.p.m*
3	4672	7.4						
7	6197	7.3	16.6	4.1	5.4	13.2	1.8	13.0
8	6450	7.1	10.0	3.3	7.1	18.0	2.7	15.1
9	6963	7.2	10.8	2.7	4.2	9.0	0.8	9.3
10	4992	6.5	17.5	5.7	11.2	44.0*	2.5	15.0
11	3680	7.1	15.5	6.0	12.3	24.6	10.1	16.0
12	8730	6.9	6.2	5.5	3.6	2.1	0.8	12.5
14	6075	7.0	9.0	5.7	13.9	22.0	1.9	11 0

* This figure is apparently too high.

The specific resistances of these soil extracts varied between 3680 ohms and 8730 ohms. Those found by Stewart (21) to be of high productivity invariably showed a lower resistance and hence a higher soluble salt content than those which produced the poorer crops. The hydrogen-ion determinations showed the soils to be about neutral, with the exception of soil 10 which was slightly acid and soils 7 and 3 which were slightly alkaline. Of the cations determined, calcium and potassium were found to vary most as between the different soils; the former from 6. 2 to 17.5 p.p.m., the latter from 3.6 to 13.9 p.p.m. of solution. Magnesium was present in smaller quantities and varied but slightly (2.7 to 5.7 p.p.m.). The phosphate-ion concentrations were less constant than had been anticipated from the recognized low solubility of soil phosphates. They varied from less than 1 p.p.m. to over 10 p.p.m. in the case of soil 11. However, with this one exceptionally high concentration, the differences were less (from a little below 1 p.p.m. to about 2.5 p.p.m.). As would be expected, nitrates were the most divergent, varying from 2 to over 20 p.p.m. The sulphate ion was the most constant of the anions. Its range was 9 to 16 p.p.m.

Comparisons between soil solutions and their aqueous extracts

Several theoretical discussions may be found in the more recent literature as to the permissibility of drawing conclusions concerning the approximate quantitative concentrations of true soil solutions from data secured by extracting these soils with pure water and comparing such results with cryoscopic determinations. While it is doubtless a fact that those substances present in the true soil solution form a certain definite fraction of those dissolved in a 1:5 pure water extract of that same soil, we are hardly able to calculate just how great a part of the solutes in such an extract formerly belonged to the true soil solution and just how much has been subsequently dissolved by the greatly diluted solution formed by the addition of so great an excess of pure water. Mitscherlich (13), and more recently Hoagland (7) (8), have attempted to compute from such extraction data the actual soil solution concentration. That many difficulties arise in such calculations have been fully realized by these investigators. For instance, Hoagland (7, p. 390) writes:

> In the first place, it is not safe to assume that a curve based on one range of extractions can accurately be extended to cover another range of extractions. Indeed, the experimental data indicate that with the smaller proportions of water the curves may change their direction very appreciably and it is unfortunately impracticable to obtain extracts for analysis in those concentrations which correspond to optimum moisture conditions. Another limiting factor previously neglected, has been described by Stewart. This concerns the differential effect of the solvent. The actual solvent in any case is not pure water, but pure water plus the solids already dissolved in the soil solution, and these vary enormously with changing conditions, even in the same soil. It is quite obvious that this factor would modify any calculations of the concentration of the soil solution based on water extracts. . . . . If we should contrast the concentration of the soil solution, calculated from the extracts to the total moisture of the soil, with the concentrations shown by the freezing-point method, there would be a general similarity of magnitude. Logically, however, a comparison is much more justifiable when the extracts are calculated not to the total soil water, but to the "free water," in the sense meant by Bouyoucos. It is then apparent that the concentration of the soil solution calculated by the extraction method is from two to five times that indicated by the depressions of the freezing point.

Hoagland, et al. (8) also remark:

> The value of the determination made by the water-extraction method rests primarily on the assumption that a logical relationship exists between water extracts and the soil solution. . . . . When a 1-to-5 extract of soil is made with distilled water, the quantity of total solids is from 1.5 to 5 times that present in the soil solution, as calculated by the frezing-point method.

That Hoagland is justified in stating that comparative data of some value may be obtained by the water-extraction procedure is generally borne out by facts hereafter presented, although results that more nearly reflect the concentration of the true soil solution might be obtained by making the freezing-point determinations at or above the soil's saturation point (16, p 229) and discarding the "unfree water" idea entirely in performing the calculations.

In order that valid comparisons may be drawn between the concentration and composition of the soil solutions and the water extracts of the same soils, it is obviously necessary to arrange the data as given in tables 7 and 9 so that they are as nearly as possible comparable. In order to gain this end, the writer has calculated all data to the basis of water-free soil; i.e., the data have been figured in such a way that the solutes in each cubic centimeter of soil solution or of water extract are the amounts dissolved from a definite weight of water-free soil. A moment's reflection will show that there are two different ways in which these data may be calculated and compared. The first would assume that in all soils a part of the soil solution is either physically or chemically combined and plays no active part as a true solvent (the "unfree-water" idea of Bouyoucos), hence it should be subtracted from the "total moisture" in the case of each soil before calculating the concentration of the soil solution in terms of "parts per million of water-free soil." The second viewpoint would assume that all of the water in soils acts as a true solvent and that none of it belongs to the so-called "unfree" class. In the light of recent work grave doubts exist in the minds of many soils scientists as to whether any such thing as "unfree water"—at least in any quantity—really exists in soils. To the writer, the presence of large amounts of "unfree water" seems highly speculative. The data has been figured both ways (see tables 10 and 11) and briefly discussed from each standpoint. The reader may choose as to the merits of each.

TABLE 10

Pressure-extracted soil solutions, with "unfree water" subtracted, compared with water-extracts of the same soils (parts per million of water-free soil)

SOIL NUMBER	CALCIUM		MAGNESIUM		POTASSIUM		NITRATE		PHOSPHATE		SULFATE	
	Soil solution	Water extract	Soil solution	Water extract	Soil solution	Water extract	Soil solution	Water extract	Soil solution	Water extract	Soil solution	Water extract
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p,p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
7	35	83	8	20	7	27	51	66	0.3	9	23	65
8	32	50	9	16	9	35	78	90	0.4	14	35	76
9	26	54	5	14	4	21	25	45	0.2	4	21	46
10	53	88	15	29	12	56	73	220*	0.4	13	26	74
11	54	78	18	29	13	62	90	123	0.7	50	35	79
12	19	31	12	27	3	18	13	11	0.3	4		63
14	30	44	8	29	13	70	82	110	0.4	9	22	55
Average..	35	62	11	23	8.5	41	59	95	0.4	15	27	65

* This figure may be in error.

Table 10 presents the comparative results, the "unfree water" (see table 6) being taken into account and subtracted.[9] Figured in this way, approximately twice as much of each of the important nutrient ions, except potassium and phosphate, are found to be extracted by an excess of solvent as was dissolved in the "free water" of the soil solutions when the latter were secured from soils at near their optimum moisture content for plant production. The averages given at the bottom of table 10 show that in the case of calcium, 35 p.p.m. are present in the soil solutions, while 62 p.p.m. are removed in the 1:5 water extracts. In the case of magnesium the ratio is 11 to 23; for nitrate,

---

[9] (Total moisture- "unfree water") × concentrations shown in table 7 = p. p. m. of dry soil.

59 to 95; and for sulfate, 27 to 65. It is interesting in this connection to note that Hoagland, discussing results secured by the cryoscopic method on these same soils, writes (7, p. 391):

> If we use the freezing point depressions as a basis, it appears that from 0.01 to 0.03 gm. of total solids is in solution for each 100 gm. of moist soil, while the total solids obtained by the 1–5 extractions vary from 0.02 to 0.06 gm. per 100 gm. of soil. This means that not more than 50 per cent could have originally been present in the soil solution. . . . . but usually the extracts would still give somewhat higher results.

As stated above, the calcium, magnesium, nitrate and sulfate comparisons (and these ions probably make up 85 to 90 per cent of the total inorganic solids) are in close agreement with these statements Unfortunately total solids were not determined on the 1:5 water extracts, so a direct comparison with Hoagland's data is not possible.

The amounts of potassium dissolved by the excess of solvent, on the other hand, are in all cases about five times as great as appear to be originally present in the soil solutions, the average ratio being 8.5 to 41 p.p.m. of dry soil. The reason for these greater amounts of potassium in the 1:5 extracts may possibly be found in the fact that these arid soils, potentially high in this element in fairly available forms, readily furnish to the excess of solvent the additional potassium required to approach equilibrium under the altered conditions.[10]

> Cameron and Bell (5) and Schreiner and Failyer (20) some years ago noted the fact that potassium (and phosphorus) behaved differently from the other soil elements in so far as solution and reabsorption phenomena were concerned. By an ingenious electrical apparatus for the continuous extraction of the water-soluble inorganic constituents of soils, these investigators showed the potassium of soil minerals to be especially soluble in pure water. By means of percolators, using both soil extracts and solutions of pure potassium salts, they were also able to demonstrate the absorption of large quantities of the potassium ion, and show that this absorptive power of soils tends largely to obscure the solvent effects. The latter investigators state:
>
> "The constancy of the removal of the absorbed potassium by water is even more striking than in the case of the phosphate and the conclusion that the true soil moisture is largely dependent on the absorptive power of the soil is well sustained by these results. The absorbed potassium is continually diffusing, and becoming directly accessible to plants."

It would appear from the above data that lesser amounts of the potassium ion are present in true soil solutions than was formerly surmised, but that in highly productive soils, the ability rapidly to renew and constantly to maintain adequate concentrations of this element obtains.

The comparative data shown for the phosphate ion are of interest. The complexity of phosphate fertilization has long been recognized. It is well known that a large excess of phosphates is necessary to maintain adequate concentrations of this element in most soils.

---

[10] We are dealing here with solution and redistribution of the K-ion between soil and solvent The colloidal surface exposed for reabsorption in these arid soils is probably small.

Schloessing, Schreiner and Failyer and others have shown that solutions obtained by successive extractions of a soil with pure water have a phosphate-ion concentration which, after one or two extractions, is practically constant, and that, when the same soil has been allowed to absorb large quantities of soluble phosphate, subsequent to the first and second leachings, the concentrations thereafter yielded are constant and practically those given by the original soil with a far less phosphate content. Schreiner and Failyer (20) thus conclude: "that the concentration of the phosphate in the soil solution is practically constant whether the soil contains a large or a small quantity of total phosphate, and that it is the absorptive power of the soil which controls this concentration in the free soil moisture. It follows that with change in the absorptive power of the soil, the concentration of the phosphate in the soil moisture would also change."

Cameron and Bell (5) have studied the effects of water and aqueous solutions upon soil phosphates and they show that an increase in the salt concentration of the solvent tends to *depress* phosphate solubility. Especially do fairly *concentrated* solutions of many of the *lime salts* notably decrease the amounts of phosphoric acid going into solution.

TABLE 11

Soil solutions and water extracts of the same soils directly compared on the basis of parts per million of water-free soil

SOIL NUMBER	CALCIUM		MAGNESIUM		POTASSIUM		NITRATE		PHOSPHATE		SULFATE	
	Soil solution	Water extract	Soil solution	Water extract	Soil solution	Water extract	Soil solution	Water extract	Soil solution	Water extract	Soil solution	Water extract
	p.p.m.	*p.p m.*	*p.p.m.*	*p.p m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
7	52	83	12	20	8	27	74	66	0.4	9	33	65
8	41	50	12	16	12	35	100	90	0.4	14	46	76
9	40	54	8	14	6	21	38	45	0.3	4	31	46
10	82	88	23	29	19	56	112*	220*	0.6	13	39	74
11	80	78	27	29	20	62	135	123	1.0	50	52	79
12	25	31	15	27	4	18	15	11	0.3	4		63
14	46	44	12	29	18	70	124	110	0.4	9	33	55
Average..	52	62	16	23	12	41	81	76	0.5	15	39	65

* One or the other of these figures is apparently in error. They have been omitted from the averages.

These facts should be borne in mind as we view the data recorded in table 10. An average of about 35 times as much phosphate ion was removed from the soils by extracting with pure water, in the proportion of 1 part of soil to 5 of water, as was present in the soil solutions expelled by direct pressure; i.e., less than one-half of one part per million (of dry soil) was present in the soil solutions, whereas about 15 p.p.m. was removed on an average with the excess of solvent. Referring to table 7 we see that the concentration of phosphate ion in the soil solutions themselves varied from 1.5 to 2.5 p.p.m. in most cases, while table 9 shows that the concentrations of the 1:5 extracts were not far from the same in p.p.m. of extract. Similar magnitudes are recorded by several investigators for the solubilities of natural tricalcium phosphate and for other soil phosphates in pure water.

Table 11 presents the same comparative data calculated directly to the dry-soil basis, on the assumption that there is no such thing as "unfree water," and that the moisture expressed simply represents fractions of uniform soil solutions, all portions of which function as true solvents.

A study of table 11 indicates that the average amounts of magnesium, calcium and nitrate ions removed by the great excess of solvent are but slightly greater than those removed in the true soil solutions.

These results are in direct accord with the recent findings of Parker (16), who shows that the concentrations of nitrate-nitrogen and of total soluble salts (figured on dry soil) are approximately the same, whether true soil solutions secured by alcohol displacement, or 1:5 water extracts are analyzed. Hoagland, et al. (8) also give data which show but slight increases in the amounts of calcium, magnesium and nitrate obtained in 1:5 water extracts over those amounts secured by 1:1 extracts. They state (8, p. 388),

> "It is plausible to assume that this diluted soil solution would bring into solution principally either 'absorbed' salts or easily soluble chemical compounds, originally derived from the more resistant minerals. . . . . This latter fraction of the soil extract would ordinarily form only a small portion of the total dissolved material."

Much doubt, it would seem to the writer, is cast on the "unfree-water" idea by the fact that only about one-half of the nitrate ion is shown to be dissolved in the soil solutions of table 10 (after subtracting the "unfree water"), while numerous data are extant showing that absorption of nitrates by soils is practically a negative quantity. Potassium ion stands in the ratio of about 1 in the soil solutions to 3½ in the extracts, while phosphate ion differs but slightly from the values shown in table 10. A possible explanation for the peculiar conduct of these two ions has already been advanced: i.e., the adsorbed potassium of soils being readily dissolved in an excess of solvent, and the sparingly soluble phosphates readily forming a saturated solution as the proportion of solvent is increased. Hoagland (8) has reported similar data.

The concentration of the sulfate ion is also greater in the water-extracts, the ratio being about 1 to 1.7. Any explanation of this fact would be purely speculative. It should be remembered, however, that the amounts of the several ions found by the analysis of these aqueous solutions are the resultants established between the processes of solution on the one hand and those of reabsorption on the other; i.e., they represent an approximate distribution equilibrium of solute between the soil and the solvent (17).

SUMMARY

The importance of more extended knowledge concerning the concentration and composition of the soil solution as it exists in soils at ordinary moisture contents is admitted by all students of our science. The present paper describes a direct-pressure method for securing sufficient quantities of this liquid phase of soils for analytical purposes. It presents a quantitative chemical study of such solutions, and finally, it compares these results with similar data from the same soils, obtained by the 1:5 water extraction method.

A somewhat detailed description of the apparatus is given. This consisted of properly constructed presses for holding the soils, while heavy testing machines supplied the required pressures.

The soils employed were fine sandy loams and came from different parts of California. Portions of these same soils had previously been used by Hoagland and Stewart in vegetation and soil-extract work (see references).

Preliminary experiments demonstrated that the pressures used had no measurable effect on soil solubility as shown by various tests made on different fractions of soil solution, secured at increasing pressures; also that there was no appreciable fining or abrasion of the soil particles due to the pressures applied.

From eight soils, moistened to but 50 per cent of their total moisture-holding capacities, over 60 per cent of the water was extracted in five cases, over 52 per cent in two cases, and about 45 per cent in the final instance. Very little solution was secured from any of these soils above 10,000 pounds pressure per square inch.

Analytical data, showing the chemical composition of the soil solutions, are submitted. This work involved determinations of Ca, Mg, K, NO_3, PO_4 and SO_4 ions, besides total solids, conductivity measurements and hydrogen-ion determinations.

Similar data are given for 1:5 water extracts of the same soils.

A comparison between soil solutions and 1:5 aqueous extracts is made, discussing the results from two points of view: First, subtracting the so-called "unfree water" in computing the concentrations of the several ions found in the soil solutions; and second, considering that all of the water in soils is free to act as a true solvent. In the light of the data submitted, the latter seems more tenable to the writer.

The important facts brought out by the above comparisons may be briefly summarized as follows: Where the "unfree water" is subtracted, about twice as much calcium, magnesium, nitrate and sulfate is removed in 1:5 water extracts as is found in the soil solutions. The potassium-ion determinations show the extracts to carry, on an average, five times as much as is dissolved in the soil solutions. In the case of phosphates, approximately 35 times as much is removed by the excess of solvent. Where we do not subtract "unfree water," but consider that all of the soil moisture is capable of uniformly dissolving soil materials, we find that the average amounts of calcium, magnesium and nitrate dissolved and removed are practically equivalent by the two methods, while about 3.5 times as much potassium and 1.7 times as much sulfate are removed by the 1:5 extraction method. The phosphate ion is still dissolved in large quantities (over 30 times as much) by the excess of solvent.

A definite relationship is shown to exist between the conductivity measurements of soil solutions and of 1:5 water extracts of the same soils. These comparative data are also in close agreement with the known productivity of the several soils examined.

All of the soil solutions are practically neutral, while the hydrogen-ion concentrations of the soils themselves vary from pH 6.5 to 7.4.

Brief mention is made of certain experiments wherein the oil-pressure method was found to be of questionable value (at least with Hawaiian soils) for securing satisfactory quantities of a uniform soil solution.

REFERENCES

(1) Bouyoucos, G. J. 1917 Measurement of the inactive, or unfree, moisture in the soil by means of the dilatometer method. *In* Jour. Agr. Res., v. 8, no. 6, p. 195–217.

(2) Burd, J. S. 1918 Water extraction of soils as criteria of their crop-producing power. *In* Jour. Agr. Res., v. 12, no. 6, p. 297–309.

(3) Burd, J. S. 1918 Chemical criteria, crop production and physical classification in two soil classes. *In* Soil Sci., v. 5, no. 5, p. 405–419.

(4) Cameron, F. K., and Bell, J. M. 1905 The universal constituents of the soil solution. U. S. Dept. Agr. Bur. Soils Bul. 30.

(5) Cameron, F. K., and Bell, J. M. 1907 The action of water and aqueous solutions upon soil phosphates. U. S. Dept. Agr. Bur. Soils Bul. 41.

(6) Ehrenberg, P., and Zyl, J. P. van. 1917 The soil solution. *In* Internat. Mitt. Bodenk., v. 7, no. 3–4, p. 141–175. (Original not seen.)

(7) Hoagland, D. R. 1918 The freezing-point method as an index of variations in the soil solution due to season and crop growth. *In* Jour. Agr. Res., v. 12, no. 6, p. 369–395.

(8) Hoagland, D. R., Martin, J. C., and Stewart, G. R. 1920 Relation of the soil solution to the soil extract. *In* Jour. Agr. Res., v. 20, no. 5, p. 381–395.

(9) Ischerekov, V. 1907 Obtaining the soil solution in an unaltered condition. *In* Zhur. Opuitn. Agron. (Russ. Jour. Exp. Landw.), v. 8, p. 147–166. (Original not seen.)

(10) Lipman, C. B. 1918 A new method of extracting the soil solution. Univ. Cal. Pub. Agr. Sci., v. 3, no. 7, p. 131–134.

(11) Loughridge, R. H. 1874 On the distribution of soil ingredients among sediments obtained in silt analyses. *In* Amer. Jour. Sci., v. 7, p. 17–19.

(12) McCool, M. M., and Millar, C. E. 1920 The formation of soluble substances in soils taken from widely separated regions. *In* Soil Sci., v. 10, no. 3, p. 219–235.

(13) Mitscherlich, E. A. 1907 Eine chemische Bodenanalyse für pflanzenphysiologische Forschungen. *In* Landw. Jahrb., v. 36, no. 2, p. 309–369.

(14) Morgan, J. F. 1916 The soil solution obtained by the oil pressure method. Mich. Agr. Exp. Sta. Tech. Bul. 28, *also in* Soil. Sci., v. 3, no. 6, p. 531–546.

(15) Northup, Z. 1918 The true soil solution. *In* Science, v. 47, p. 638–639.

(16) Parker, F. W. 1921 Methods of studying the concentration and composition of the soil solution. *In* Soil Sci., v. 12, no. 3, p. 209–232.

(17) Patten, H. E., and Waggaman, W. H. 1908 Absorption of soils. U. S. Dept. Agr. Bur. Soils Bul. 52.

(18) Ramann, G., März, S., and Bauer, H. 1916 The soil solution obtained by the action of a hydraulic press. *In* Internat. Mitt. Bodenk., v. 6, p. 27; *abs. in* Chem. Abs., v. 11, p. 3078, and *in* Exp. Sta. Rec., v. 38, p. 5–12. (Original not seen).

(19) Saussure, T. de 1804 Recherches chimiques sur la Vegetation, p. 168–9. Paris.

(20) Schreiner, O., and Failyer, G. H. 1906 The absorption of phosphates and potassium by soils. U. S. Dept. Agr. Bur. Soils Bul. 32.

(21) Stewart, G. R. 1918 Effect of season and crop growth in modifying the soil extract. *In* Jour. Agr. Res., v. 12, no. 6, 311–368.

(22) Taylor, E. McK. 1919 An investigation of soil water. Essex Ed. Com., East Anglian Inst. Agr. Bul. 25.

(23) Zyl, J. P. Van. 1916 The soil solution, how it is obtained, its composition and use in mechanical analysis by sedimentation. *In* Jour. Landw. v. 64, no. 3, p. 201–275; *abs. in* Expt. Sta. Rec., v. 36, p. 720. (Original not seen.)

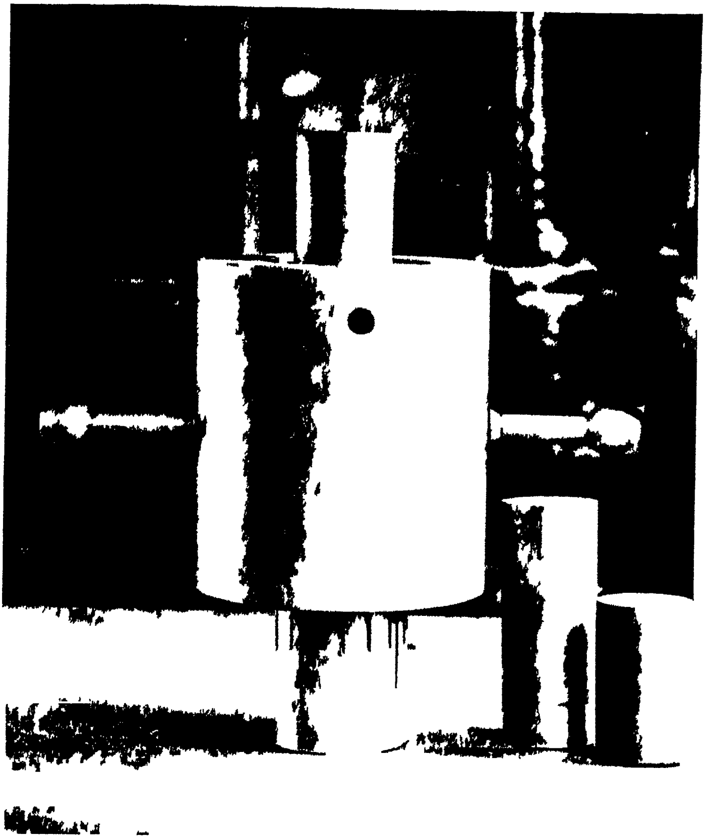
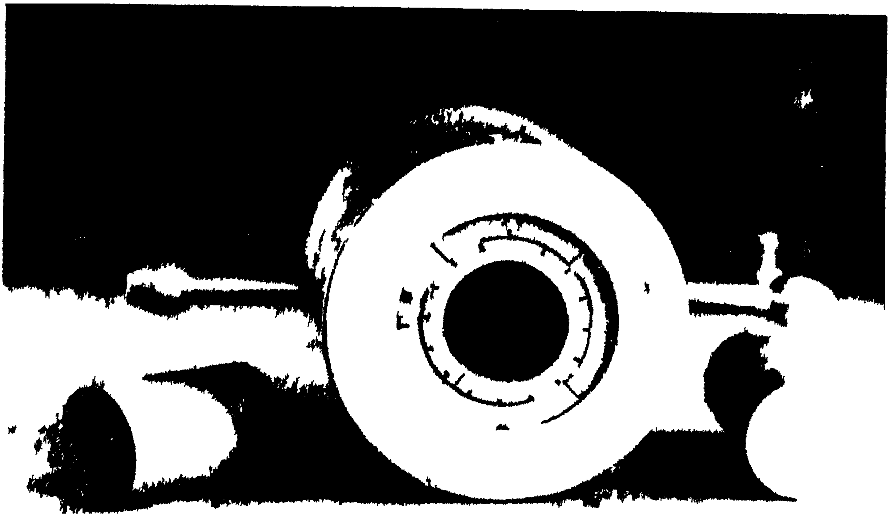


Fig 1 Press No 2, Side View



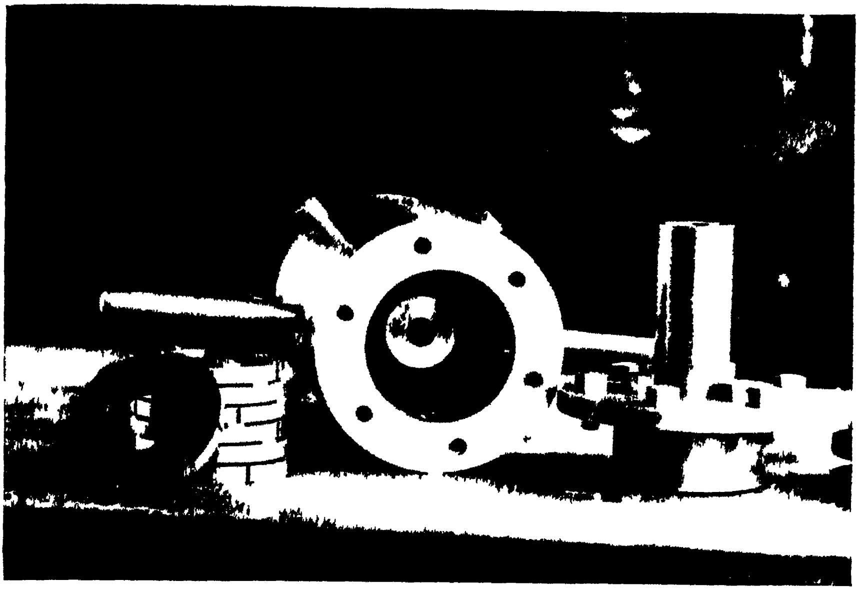


FIG. 1. PRESS NO. 3, SHOWN IN DETAIL

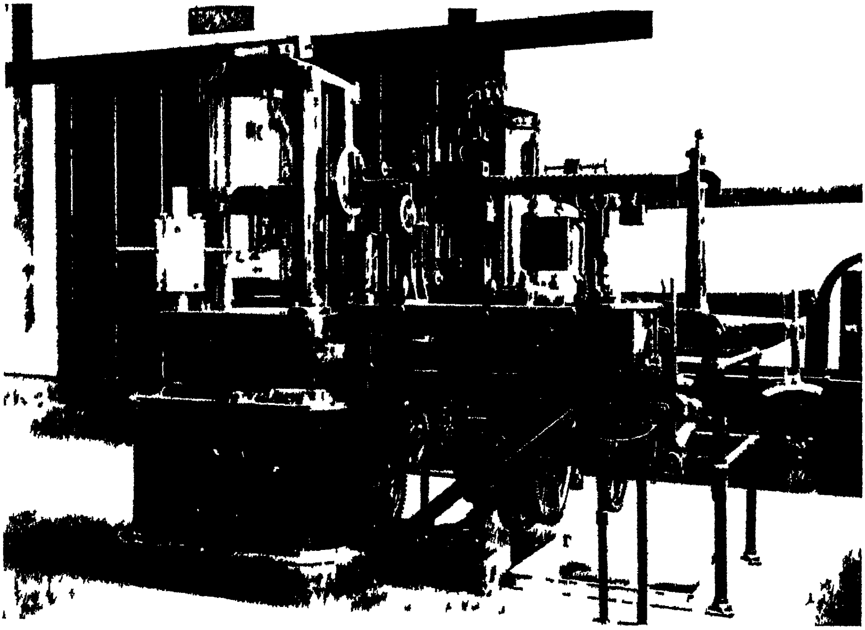


FIG. 2. PRESS NO. 3, ASSEMBLED AND RESTING ON TABLE OF TESTING MACHINE

A NOTE ON OXIDATION OF SULFUR IN OREGON SOILS[1]

J. S. JOFFE AND H. C. McLEAN

New Jersey Agricultural Experiment Stations

Received for publication February 17, 1921

As outlined elsewhere (1,2) the sulfur transformations in soils consists in oxidation of sulfides, sulfites and elementary sulfur to sulfates. Since sulfides have to go through the sulfur stage, a study of sulfur oxidation naturally includes sulfide oxidations. The soil is the medium of these reactions. Their successful accomplishment depends, therefore, upon the fitness of the soil for the proper functioning of the organisms. Since soils in close proximity may vary in composition, texture, drainage, temperature, reaction, etc.; these conditions determine for each soil a certain capacity to oxidize sulfur. Some of these features have been studied before, as may be seen from the publications referred to (1,2), but the relation of capacity of soils to adjust their reaction to changes in intensity of acidity due to sulfur oxidation, the capacity to absorb or adsorb the acids formed, the differences in oxidation from inoculated and uninoculated has not been dealt anywhere.

The experiments reported below were undertaken to investigate these features on a series of 8 Oregon soils. These soils are known for their response to sulfur treatments with consequent increases in alfalfa yields, the increases varying from 35 to 500 per cent.

The soils here described were obtained from the Oregon Experiment Station through the courtesy of Dr. Reimer, who describes these soils in Bulletin 163 of the Oregon Agricultural Experiment Station (4) in connection with the use of sulfur as a fertilizer for alfalfa:

1. Agate gravelly loam. A red, clay loam underlaid with an impervious hard pan. The surface soil contains 0.024 per cent of sulfur. Sulfur applications proved very beneficial.
2. Tolo loam. Typical red, foothill clay loam, underlaid with a tenacious yellow clay. Sulfur applications was slightly beneficial.
3. Medford loam. A deep brown fertile silt loam; the soil contains .036 per cent of sulfur; best alfalfa soil in Rogue River Valley; no benefits from sulfur applications.
4. Antelope clay adobe. Heavy black adobe soil of a very sticky nature, deep and well drained; the sulfur content is 0.02 per cent. Sulfur application increased the yield as high as 1000 per cent.
5. Phoenix clay adobe. Heaviest adobe, contains 63 per cent of clay and 21 per cent of silt; the sulfur content is 0 021 per cent; iron sulfate had no effect on yields.

---

[1] Paper No. 83 of the Journal Series New Jersey Agricultural Experiment Station, Department of Soil Chemistry and Bacteriology. This paper will appear in RUTGERS COLLEGE STUDIES, vol. 1.

6. Barren coarse sand. Coarse granite of considerable depth; the sulfur content is 0.028 per cent; sulfur applications proved beneficial.

7. Anderson clay loam. Deep, black fertile clay loam of alluvial origin. No response to sulfur applications.

8. Salem clay loam. Deep fertile clay loam, contains 21 per cent of clay and 40 per cent of silt; the sulfur content is 0.027 per cent; sulfur applications increased the yield as high as 500 per cent.

EXPERIMENT 1

One-hundred-gram portions of these soils were distributed in tumblers, 100 mgm. of sulfur[2] was added to each one and sufficient water to make up the moisture optimum for each particular soil. Two series were prepared in one inoculated sulfur was used, in the other uninoculated.[3] Table 1 gives the results of the experiment.

TABLE 1

Course of reaction and amount of sulfur oxidized

Sulfur application, 2000 pounds per acre

SOIL NUMBER	REACTION OF ORIGINAL SOIL	INOCULATED SULFUR				UNINOCULATED SULFUR			
		After 15 days' incubation		After 32 days' incubation		After 15 days' incubation		After 32 days' incubation	
		Reaction	Sulfur oxidized in 100 gm. of soil	Reaction	Sulfur oxidized in 100 gm. of soil	Reaction	Sulfur oxidized in 100 gm. of soil	Reaction	Sulfur oxidized in 100 gm. of soil
	pH	*pH*	*mgm.*	*pH*	*mgm.*	*pH*	*mgm.*	*pH*	*mgm.*
1	6.2	6.2	10.39	5.1	43.95	6.2	6.01	5.2	30.09
2	6.2	6.0	37.98	5.8	58.25	6.0	32.73	5.8	60.99
3	6.2	6.0	23.68	5.2	61.52			5.2	56.06
4	6.4	6.0	8.48	5.2	41.39	6.4	8.2	5.4	31.17
5	6.4	6.0	24.61	5.8	44.2	6.4	5.47	6.0	38.77
6	6.0	5.2	33.15	4.4	54.75	6.0	23.85	4.4	56.94
7	6.2	5.4	34.74	5.4	64.26	5.8	31.18	5.6	43.33
8	6.4	6.0	19.14	5.4	56.83	6.0	22.7	5.2	63.18

The first striking feature of the data is the specificity of each soil as a medium for the sulfur oxidizing flora, i.e., the inherent sulfur-oxidizing capacity of the soil. It will be noticed that soil 4, after 15 and 32 days of incubation, oxidized less sulfur than any other soil. The next lowest in sulfur oxidizing capacity was soil 1. An examination of the physical character of these two soils will show that they are heavy in texture and consequently not well aerated. This condition, is indispensable for efficient sulfur oxidation. These soils, however as reported by Reimer and Tartar (4), have responded favorably to sulfur treatment, with increases in crop production as high as 1000 per cent. Reimer was fully justified in recommending that the sulfur be applied far in advance of the growing season. Such a practice develops an active sulfur oxidizing flora which has to adjust itself to the adverse conditions of aeration. In speaking of sulfur as a fertilizer it has been mentioned that as yet the economy of sulfur metabolism by plants is not established. It is possible that large quantities of sulfates have to come into immediate contact with the roots before they can absorb the little sulfur the plant needs. From this standpoint, it is desirable to get as active oxidation as possible. The abundance of sulfates formed in the advanced periods of incubation will suffice for both leaching and crop uses. It is of interest to note that after 15 days of incubation the soils which did not respond so well to sulfur applications oxidized more sulfur than those that did benefit by its application. This was also true after 32 days of incubation. The only explanation that may be offered is that the soils not deficient in sulfur are perhaps in a better physical condition, as it may be inferred from the descriptions.

---

[2] The sulfur added was calculated to be equivalent to 2000 pounds per acre of 2,000,000 pounds of soil.

[3] Through an error, the uninoculated soils were stirred with the same spatulae as the inoculated soils on the tenth day of incubation. Due to lack of material the experiment could not be repeated at this point.

Another interesting feature is the capacity of these particular soils to neutralize, absorb and adsorb the acid formed. The buffer content of these soils seems to be fairly high. Especially is this true with the heavy soils. The record of the hydrogen-ion concentration measurements may serve as an index of the buffers present. It will be noticed that after 15 days of incubation the change in the pH values was only slight, although as high as 37.98 per cent of the sulfur was oxidized in soil 2. Since the application of sulfur was on the basis of 2000 pounds per acre it is clear that some soils will tolerate and benefit by unusually large applications of sulfur and not be injured even if there is a sufficient balance of this essential element in the particular soil. Soil 3 may serve as an illustration. According to Reimer and Tartar (4), this is one of the best alfalfa soils in Rogue River Valley. It is well supplied with sulfur in the proper form and sulfur applications did not increase the yield of alfalfa. But data show that in 15 days 23.68 per cent or 473 pounds of sulfur, was oxidized and still the reaction changed but slightly. Soil 7 oxidized close to 700 pounds of sulfur per acre in 15 days, but its buffer power is not as high as that of soil 3 and the reaction went down to a pH 5.4. This would indicate that for this soil a 700 pound application would be somewhat too much; from the pH values and the amount of sulfur oxidized one may easily predict the amount of sulfur it is safe to apply to any of the Oregon soil studied. The same criterion may be applied to every soil that is poor in sulfur. Soil 6 was a sandy soil having a very little buffering effect and naturally showed acidity at high applications of sulfur, although it did respond to small applications of sulfur as pointed out by Reimer. After 15 days it oxidized over 660 pounds of sulfur and the pH was 5.2, which is not a favorable reaction for the best growth of alfalfa. In such cases a heavy application of sulfur will show harmful effects, although a judicious application would undoubtedly be beneficial, as in the case of soil 6.

The third feature of this experiment is the difference in the speed of oxidation of sulfur in the inoculated and uninoculated soils. As pointed out the experiment could not be carried out as it was planned: the uninoculated soils were subject to inoculation after ten days. Still the differences are striking. In most cases the inoculate tumblers showed greater sulfur oxidation. Of especial interest is soil 5, the inoculated series of this soil oxidized in the first 15 days nearly five times as much sulfur as the uninoculated. The practical inference is that inoculated sulfur may be applied later and thus prevent losses by leaching. Moreover smaller amounts may be applied. This corroborates the results of Martin (3). It may be of interest to note that after a more prolonged incubation period nearly all the sulfur was oxidized and the reacton of the first five soils did not go below pH 5.0; soils 4 and 5 had a pH 5.4. This indicates the enormous capacity of these soils to oxidize sulfur and still maintain a reaction which will not inhibit growth, even of alfalfa.

TABLE 2

Course of reaction and amount of sulfur oxidized

Sulfur application, 250 pounds

SOIL NUMBER	PERIOD OF INCUBATION				
	15 days	30 days	45 days	60 days	
				Reaction	Sulfur oxidized
	pH	*pH*	*pH*	*pH*	*per cent*
1	6.2	6.0	6.0	6.0	72.5
2	6.2	6.2	6.0	6.2	81.6
3	6.2	6.0	6.2	6.0	84.2
4	6.2	6.2	6.2	6.0	71.4
5	6.4	6.2	6.2	6.2	74.9
6	5.8	5.6	5.6	5.3	86.3
7	6.0	5.8	5.8	5.8	87.2
8	6.2	6.0	6.0	6.0	82.3

EXPERIMENT 2

To further test the buffer capacity of these soils, the following experiment was carried out. The same procedure was used as in the first experiment. Only inoculated sulfur was used in this experiment. Sulfur was applied at the rate of 250 pounds per acre. Table 2 of the pH values shows that after a period of 60 days practically no change in the hydrogen-ion concentration took place. Every soil except soil 6 accomodated this amount very much to its advantage and enriched its sulfur resources. To see if the sulfur was oxidized, sulfates were determined after 60 days incubation and 70 to 80 per cent of the sulfur appeared as sulfates. Some sulfur was perhaps adsorbed as sulfates or in some other form. The data given in table 2 show that more than 250 pounds of sulfur may be applied to the soils studied without imparting a reaction inhibitory to the successful growth of alfalfa.

SUMMARY

1. The sulfur oxidizing capacity of 8 Oregon soils was investigated. It was apparent that the soils of a low sulfur oxidizing capacity should receive the sulfur application in advance of the growing season.

2. The buffer action of these soils was measured by the changes in the hydrogen-ion concentration of the soil extract. It was found that most of the soils investigated did not materially change in reaction after having oxidized most of the 250 pounds of sulfur used, thus indicating that elemental sulfur may be applied as a fertilizer to these soils without danger of injuring crop production due to the acidity of sulfuric acid formed.

3. Comparisons of inoculated and uninoculated sulfur show that the former is more effective.

REFERENCES

(1) Joffe, Jacob, S. 1922 Studies of sulfur oxidation in sulfur-floats-soil mixtures. *In* Soil Sci., 13, v. p. 107–118.

(2) Joffe, Jacob, S. 1922 Some agricultural relations of sulfur. Thesis, Rutgers College.

(3) Martin, W. H. 1921 A comparison of inoculated and uninoculated sulfur for the control of potato scab. *In* Soil Sci., vol. 11, p. 75–85.

(4) Reimer, F. C., and Tartar, H. V. 1919 Sulfur as a fertilizer for alfalfa in south Oregon. Ore. Agr. Exp. Sta. Bul. 163.

THE POTENTIAL ACIDITY OF SOILS

OLOF ARRHENIUS[1]

University of California

Received for publication November 30, 1921

Since Bjerrum (5) in his first two papers in a series on the causes of actual and potential[2] acidity of soils pointed out the great theoretical value of the knowledge of these properties, very few have worked on this subject.

The author, when investigating these soil characters, especially on some Oriental soils (1, 3, 4) found results which may be of both theoretical and practical value.

Chiefly, there are two different methods for measuring hydrogen-ion concentration, viz., electrometric and colorimetric. All measurements in this work are made according to the colorimetric method as modified by Gillespie (8). The accuracy of this method to the first decimal is quite sufficient for soil investigations where the deviations are so great, and it is so convenient that it can be used under conditions where no laboratory equipment is available.

The hydrogen-ion concentration was measured in soil extract made up as follows:

Ten grams natural moist soil were shaken with 50 gm. distilled water and then left to settle. The next day the extract was filtered and the pH value of the filtrate was determined. By this method there was introduced a source of error, viz., the probable change in the pH value of the soil solution when diluted. Sharp and Hoagland (14), however, give experimental data for the correctness of this method when not too large amounts of water are used.

The potential acidity in the following experiments was measured as follows:

In a series of flasks, usually nine, 5.00 gm. soil was introduced into each and then 10, 5, 2 and 1 cc. of about 0.1 *N* acid, or the same amounts of alkali was added, except in the case of the check, and the solution made up to 20 cc. with distilled water. The flasks were then corked, shaken for a time and left to settle. The whole procedure takes one day. After filtering, the pH values were determined colorimetrically in the filtrate. The amount of acid or alkali added is calculated in cc. of 0.1 *N* solution per gram of dried soil 100° C.

Sometimes stored soils have been used for hydrogen-ion concentration investigations and in order to determine the influence of drying on both the actual and potential acidity, the following experiment was carried out.

---

[1] Most of the work reported here has been carried out in the soils and bacteriology laboratory of the University of California. The author wishes to take this opportunity to thank Professors C. B. Lipman, W. F. Gericke, A. R. Davis, and D. R. Hoagland for their kind help and advice.

[2] Here actual acidity means the same as hydrogen-ion concentration. Potential acidity means the changes in actual acidity obtained when acids or alkalies are added.

A humus-rich soil was put into four different beakers. One was covered with a glass and kept in the dark and at constant temperature; one was dried at room temperature (varying between 15° and 20°C.); one was dried at 100°C. and one at 150°C. After 14 days, the titration curves were made. Results are shown in table 1. From this it is seen that within the experimental error the results are quite congruent and the different treatment did not influence the acidity of the soil.

TABLE 1

The influence of drying on soil acidity

KIND OF SOIL		TREATED WITH 0.1 N H_2SO_4				TREATED WITH 0.1 N NaOH				
A. Natural moist soil.........	*cc.*	*2.50*[*]	*1.25*	*0.50*	*0.25*	*0.00*	*0.13*	*0.26*	*0.65*	*1.2*
	pH	4.3	5.2	6.2	7.0	7.2	7.3	7.3	7.4	8.0
B. Air dried 15–20°C..........	*cc.*	*2.62*	*1.51*	*0.52*	*0.26*	*0.00*	*0.13*	*0.27*	*0.67*	*1.33*
	pH	4.0	4.8	6.3	7.0	7.2	7.2	7.3	7.4	8.0
C. Steam dried 100°..........	*cc.*	*2.60*	*1.30*	*0.52*	*0.26*	*0.00*	*0.13*	*0.27*	*0.66*	*1.33*
	pH	4.1	5.3	6.3	7.0	7.2	7.3	7.3	7.4	8.0
D. Heat dried 150°...........	*cc.*	*2.50*	*1.25*	*0.50*	*0.25*	*0.00*	*0.13*	*0.26*	*0.64*	*1.27*
	pH	4.3	5.3	6.3	7.0	7.2	7.3	7.3	7.4	8.0

[*] In this, as well as tables 2 and 5–9, figures in italics show the amount of acid or alkali which has been added to the soil while the regular type shows the resulting reaction.

TABLE 2

The influence of different acids and alkalies on soil reaction

ACID ADDED		TREATED WITH 0.1 N ACID				ALKALI ADDED		TREATED WITH 0.1 N ALKALI				
A. H_2SO_4.......	*cc.*	*2.42*	*1.21*	*0.48*	*0.24*	NaOH	*cc.*	*0.00*	*0.12*	*0.25*	*0.61*	*1.22*
	pH	3.1	4.1	5.0	6.2		pH	7.0	7.4	7.8	8.4	9.3
B. HCl.	*cc.*	*0.43*	*0.22*	*0.11*	*0.05*	KOH	*cc.*	*0.00*	*0.04*	*0.08*	*0.17*	*0.34*
	pH	5.7	6.3	6.6	6.8		pH	7.0	7.4	7.6	8.0	8.5
C. HNO_3.......	*cc.*	*0.32*	*0.16*	*0.08*	*0.05*	H_4NOH	*cc.*	*0.00*	*0.05*	*0.08*	*0.16*	*0.32*
	pH	0.9	6.3	6.7	6.9		pH	7.0	7.2	7.5	7.8	8.1
D. CH_3COOH...	*cc.*	*0.35*	*0.18*	*0.09*	*0.05*	$Ba(OH)_2$	*cc*	*0.00*	*0.05*	*0.06*	*0.20*	*0.41*
	pH	5.0	5.5	5.9	6.9		pH	7.0	7.4	7.6	8.0	8.5
After 3 days.....	pH	(5.6)		(6.0)			pH					

A short storage, therefore, as for transportation etc., does not interfere with the work, but one must be very careful with soils stored for a long time.[3]

Another objection is that one may obtain different results when using different acids or alkalies. In order to control this the titrations were made in four series, using H_2SO_4, HCl, HNO_3 and CH_3COOH, as acids, and NaOH, KOH, NH_4OH and Ba $(OH)_2$ for the alkalies. The results of these tests are given in table 2, which shows that within the limits of experimental error, equivalent amounts of acid or alkali caused equal changes except in the case of acetic acid which gave a higher acidity in the filtrate. But when the soil is left in contact with the acetic acid for a period of three days, the same results are obtained because of the low dissociation of the acetic acid and thus lower rate of action.

---

[3] In the case of the Java or Egyptian soils, where stored soils also were employed in the work, tests with the fresh field soil gave the same results as the stored ones.

Before discussing the importance of the potential acidity, a short review must be given of the influence of hydrogen-ion concentration on most cultivated plants.

When the pH value is over 9 or under 4, growth is inhibited so that only a few species are able to grow there without being severely injured.

Such results were obtained in culture experiments by Hoagland (10) and others (13). As all these workers have made their experiments with either solution or sand cultures and as the conditions in these media probably are not quite natural, the author undertook a similar work with soil.

The soil employed is garden soil from Java (table 7). Eight pots were filled with this soil and they were treated with different amounts of acid and alkali so that a series of soils was obtained whose pH values were whole numbers and ranged from 3 to 10. Table 3 shows the effect of this treatment on germination, stand after 2 months, average leaf surface after 14 days and 2 months, and the total leaf surface after 2 months. These results show a close resemblance to those obtained either in sand or in water cultures. It is evident that the optimum hydrogen-ion concentration for all plants investigated lies within a wide range.

Within this optimum range, we find a mass of irregularities in the growth of plants which depend upon the influence of the actual acidity.

The bacterial flora (7) of the soil plays a very great rôle in the nutrition balance and life of higher plants. So, for instance, we have the different nitrifying bacteria working in different pH zones.

Some of the plant pathogenes[4] spend a part of their life cycle in the soil and are dependent on the hydrogen-ion concentration of the substrata; others are not, as is easily seen from table 4.

Probably the fauna of the soil also is strongly influenced by this factor as has been shown for earthworms (2).

The solubility of different salts is also dependent on the degree of acidity of the soil solution. For instance, iron phosphate and aluminum phosphate are precipitated at a pH value of 3 to 5. The same thing happens with other phosphates and calcium and magnesium salts, as is seen from the titrations by Hildebrand (9). In the case of aluminum salts a large amount of these in solution may cause a poisoning of plants, as is shown for rye and rice.

---

[4] The strains were kindly furnished by Prof. Horne, Univ. of Cal.

TABLE 3

The influence of soil acidity on plant growth

SOIL REACTION	RATE OF GERMINATION	GERMINATION	LIVE PLANTS AFTER 2 MONTHS	AVERAGE LEAF SURFACE		TOTAL RELATIVE LEAF SURFACE AFTER 2 MONTHS
				14 days	2 months	
			Bersim			
pH		*per cent*	*per cent*			
3	0.06	15	0	0	0	0
4	0.20	47	25	1.7	2.8	0.35
5	0.20	60	51	2.9	7.2	2.2
6	0.32	62	55	4.8	4.7	1.6
7	0.42	75	78	6.8	7.1	4.2
8	0.47	82	71	8.3	11.2	6.5
9	0.66	100	73	8.5	6.6	4.9
10	0.25	57	50	7.6	2.7	0.75
			Barley			
3	0.13	65	72	21	39	18.3
4	0.23	95	71	27	36	24.1
5	0.18	97	66	29	53	33.4
6	0.29	95	100	29	63	59.8
7	0.25	100	90	30	67	60.3
8	0.31	97	74	34	65	46.8
9	0.31	100	93	32	80	74.4
10	0.26	100	54	30	38	20.5
			Corn			
3	0.10	67	100	24	41	31.6
4	0.15	95	100	28	65	61.7
5	0.16	95	98	26	56	52.0
6	0.16	93	98	33	79	71.0
7	0.24	100	98	31	53	51.9
8	0.23	95	100	40	56	53.2
9	0.24	100	100	44	58	58.0
10	0.25	100	98	54	67	65.7
			Cotton			
3	0.07	50	57	22	17	4.6
4	0.14	62	86	41	67	34.8
5	0.20	72	91	82	68	44.2
6	0.18	82	94	83	75	56.2
7	0.23	82	88	82	71	51.1
8	0.23	85	98	74	50	41.5
9	0.19	75	90	101	79	53.6
10	0.18	82	76	4	24	14.9

TABLE 3—*Continued*

SOIL REACTION	RATE OF GERMINATION	GERMINATION	LIVE PLANTS AFTER 2 MONTHS	AVERAGE LEAF SURFACE		TOTAL RELATIVE LEAF SURFACE AFTER 2 MONTHS
				14 days	2 months	
			Wheat			
pH		*per cent*	*per cent*			
3	0.05	17	100	11	26	3.4
4	0.11	37	87	12	24	7.6
5	0.24	52	94	33	63	30.5
6	0.28	55	100	28	53	29.0
7	0.28	72	91	25	50	32.8
8	0.29	72	88	47	80	50.4
9	0.35	70	70	50	86	41.0
10	0.17	42	100	7	27	11.0

When working with different tropical and subtropical soils the author found a very good correlation in some cases between the fertility of the soil and the titration curve. When the soils showed a strong buffer effect,[5] they were of good fertility; when they acted as weak buffers, their fertility was poor, (see tables 5–8).

Table 5 represent 16 tobacco soils from Sumatra. The rest are grouped around the four here given. Those acting as good buffers and more alkaline than pH 7 are resistant against the tobacco-wilt disease (Slijmziekte, *Bact. solanacearum*). The weak buffer soils, and those more acid than pH 7, however, are often devastated by this disease. In the weak buffer soils, the reaction is easily changed into the optimum field of bacterial growth.

The investigations of 20 rice soils (4) from Java are represented by two soils (in table 6), Soere and Rimboelor. Here we find the same conditions as in the former case, probably we have to deal with a weakening of the rice plant and possibly with the effect of aluminum. Of great interest is that these two soils are from the same district and of the same origin, but afterwards

TABLE 4

Influence of hydrogen-ion concentration on viability of plant pathogenes

NAME OF ORGANISM	INDEX OF VIABILITY AT DIFFERENT SOIL REACTIONS						
	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9	pH 10
Bacterium citriputeale	0	2	8	10	10	10	5
Botrytis cinerea (potatoes)		8	7	5	4	3	2
Pythium sp. (potatoes)		10	10	10	10	5	10
Sclerotium rolfsii (Iris)	8	8	9	9	10	10	10
Polystictum versicolor	0	10	10	10	10	6	6
Bact. solanacearum (tobacco)	0	5	10	6	0		

---

[5] By buffer effect is meant the ability to take up acids or alkalies without or but slightly changing the actual acidity.

differentiated by several factors. In the same table we find the result of a titration of a humus-rich garden soil and of a volcanic ash, taken fresh and stored for two years. It may be of interest to compare this young soil with the relatively old Demak soils.

The titration curves of some Egyptian (1) soils are found in table 7. Of these the Damanhour soil is exceedingly unfertile, the Nile suspension and the garden soil, however, are very fertile. In this case we see that the pH value of the Damanhour soil lies on the alkaline side of the growth limit. The sand is a soil type which is easily reclaimed, but also very easily destroyed by alkali. Also in these soils we see the same thing as in the case of the volcanic ash and the Demak soils. The Nile suspension has a comparatively weak buffer action when compared with the old garden soil and Damanhour soil.

TABLE 5

Effect of addition of acid or alkali upon the hydrogen-ion concentration of tobacco soils (Sumatra)

SOIL		TREATED WITH 0 1 *N* H_2SO_4				TREATED WITH 0 1 *N* NaOH				
Kaloean Penang Black, good....	*cc.*	*2.80*	*1.40*	*0.56*	*0.28*	*0.00*	*0.29*	*0.58*	*1.46*	*2.92*
	pH	4.0	5.0	7.0	7.2	7.2	7.4	7.6	8.4	9.5
Songei Mentjirim Black, bad...	*cc.*	*2.49*	*1.25*	*0.50*	*0.25*	0 00	*0.26*	*0.52*	*1.30*	*2.59*
	pH	2.5	3.5	4.5	5.5	7.0	7.5	8.0	>8.0	>8.0
Toentongan Black, good.......	*cc.*	*2.55*	*1.28*	*0.51*	*0.26*	*0.00*	*0.27*	*0.53*	*1 33*	*2.66*
	pH	4.0	5.0	6.0	6.8	7.0	*7.2*	7.5	8.0	>8.0
Toentongan Red, bad.........	*cc.*	*2.41*	*1.20*	*0.42*	*0 24*	*0.00*	*0.25*	*0 50*	*1 25*	*2.50*
	pH	2.0	3 0	4 0	5 0	6.7	7.2	8 0	>8 0	>8.0

> This sign indicates "more than."

TABLE 6

Effect of addition of acid or alkali upon the hydrogen-ion concentration of Java soils

SOIL		TREATED WITH 0 1 *N* HCl				TREATED WITH 0.1 *N* NaOH				
Rimboelor Demak, good..........	*cc.*	*2.33*	*1.17*	*0.47*	*0.23*	*0.00*	*0.14*	*0.27*	*0.68*	*1.37*
	pH	6.5	7.2	7.7	7.8	7.8	7.9	8.1	9.0	10.0
Soere Demak, bad..............	*cc.*	*2.44*	*1.22*	*0.49*	*0.24*	*0.00*	*0.14*	*0.28*	*0.71*	*1.42*
	pH	3.8	5.0	6.5	6.7	7.0	7.2	7.4	8.8	9.8
Garden soil Buitenzorg, good	*cc.*	*4.8*	*4.24*	*2.94*	*2.12*	*0.84*	*0.42*	*0.00*	*0.24*	*0.50*
	pH	4.5	6.5	6.8	7.0	7.2	7.3	7.3	7.6	7.9
								1.24	*1.68*	*2.48*
								8.8	9.5	11.0
Volcanic ash Kediri.............	*cc.*	*2.12*	*1.06*	*0.41*	*0.20*	*0.00*	*0.12*			
	pH	2.6	3.5	4.0	4.5	7.0	9.5			

Among the California soils the strongest buffer effect was shown by the garden soil.

The soils 1, 3 and 6 (used by Stewart (15)) are of the same soil type and show similar titration curves, whereas number 7 is of another type; here, however, 3 and 7 are bad and 1 and 6 good. In this case the pH value is at the neutral point and apparently the changes in reaction caused by the plants do not form any unfavorable conditions, whereas other factors, as the amounts of nutrients present are the chief factors of growth.

Of great interest are the soils from Ignacio near San Francisco. One of the samples, an extremely acid subsoil, probably represents the soil type before being worked and limed. The unlimed soil is thoroughly worked, but not limed and is now yielding a poor crop of beets; on the same field a part was limed resulting in an exceedingly good crop. The value of the soils is quite in accordance with what has been said above on the correspondence between titration curve and soil fertility.

TABLE 7

Effect of addition of acid or alkali upon the hydrogen-ion concentration of Egyptian soils

SOIL USED		TREATED WITH 0.1 *N* HCl			TREATED WITH 0.1 *N* NaOH				
Garden soil Giza, good	*cc.*	*3.17*	*1.98*	*0.75*	*0.00*	*0.29*	*1.02*	*2.08*	
	pH	7.2	7.4	7.5	7.6	8.0	9.5	11.0	
Damanhour, soil, bad	*cc.*	*0.00*	*0.31*	*0.72*	*1.15*	*1.56*	*2.15*	*3.71*	*5.4*
	pH	11.00	10.0	9.0	8.7	8.4	8.1	7.6	7.3
Nile Suspension, Cairo	*cc.*	1.77	*0.93*	*0.11*	*0.44*	*0.20*	*0.00*	*0.23*	*0.47*
	pH	3.0	6.5	7.0	7.2	7.4	7.4	7.5	7.8
Sand El-Munayer	*cc.*	*0.29*	*0.00*	*0.20*	*0.32*	*0.76*		*1.25*	*1.94*
	pH	4.0	7.0	7.4	7.8	11		9.2	10.5

From the tables one sees that there is a correlation between the buffer action and the fertility of the soil. If the actual acidity of a soil lies within certain limits the buffer action of the soil is of great importance, so that strong buffer action is a good property; weak, a bad property. This action, however, is an indirect one. All soils are in a developing phase. The vegetation plays a great rôle on the one hand through the decomposing plant debris and root excretions and on the other hand through substances formed by the decomposition such as carbonic acid, etc. In arid climates the soil is more influenced by the formation of alkaline substances.

If there is, for instance, an acidifying process going on in the soil we may look at the process as a sort of a titration with an acid. A certain amount of acid substances is given off by the plant and in some soils we see a strong change caused by this action, in others the change is perhaps scarcely visible. The latter soils are the strong buffers, the former, weak. In this way the soil reaction of the weak buffer soils is more easily changed to a range which is in some way injurious to the plant, than in the case with those having a strong buffer effect. Then the question arises how to improve the buffer effect of soil.

TABLE 8

Effect of addition of acid or alkali upon the hydrogen-ion concentration of California soils

SOIL USED		TREATED WITH 0.1 *N* H_2SO_4					TREATED WITH 0.1 *N* NaOH			
1A	*cc.*	*2.42*	*1.21*	*0.48*	*0.24*	*0.00*	*0.12*	*0.25*	*0.61*	*1.22*
	pH	3.1	4.1	5.0	6.2	7.0	7.4	7.8	8.4	9.3
1B	*cc.*	*2.29*	*1.15*	*0.45*	*0.23*	*0.00*	*0.11*	*0.23*	*0.58*	*1.17*
	pH	3.3	4.5	5.4	5.9	7.0	7.4	7.8	8.1	9.0
3A	*cc.*	*2.60*	*1.30*	*0.52*	*0.26*	*0.00*	*0.13*	*0.27*	*0.67*	*1.33*
	pH	3.0	4.3	5.1	6.0	7.0	7.4	8.2	9.0	10.0
3B	*cc.*	*2.25*	*1.12*	*0.45*	*0.23*	*0.00*	*0.12*	*0.24*	*0.57*	*1.14*
	pH	3.3	4.8	5.8	6.2	7.0	7.4	7.8	8.4	9.5
6A	*cc.*	*2.61*	*1.30*	*0.52*	*0.26*	*0.00*	*0.12*	*0.27*	*0.66*	*1.32*
	pH	2.9	4.4	5.6	6.2	7.0	7.6	8.1	9.0	10.5
6B	*cc.*	*2.35*	*1.17*	*0.47*	*0.24*	*0.00*	*0.12*	*0.25*	*0.60*	*1.19*
	pH	3.2	4.8	5.9	6.4	7.0	7.5	7.8	8.3	9.7
7A	*cc.*	*2.34*	*1.17*	*0.47*	*0.23*	*0.00*	*0.12*	*0.23*	*0.59*	*1.19*
	pH	1.7	2.8	4.4	5.4	6.7	7.0	7.9	8.7	9.0
7B	*cc.*	*2.16*	*1.08*	*0.43*	*0.22*	*0.00*	*0.11*	*0.22*	*0.55*	*1.10*
	pH	2.0	3.0	4.6	5.4	6.7	7.0	7.7	8.7	9.5
Garden soil	*cc.*	*4.44*	*2.22*	*1.11*	*0.44*	*0.22*	*0.00*	*0.05*	*0.11*	*0.57*
	pH	2.5	4.8	5.2	6.2	7.0	7.2	7.3	7.3	7.4
									1.13	*4.52*
									8.0	12.0
Ignacio Hard, Subsoil	*cc.*	*3.04*	*1.52*	*0.61*	*0.30*	*0.0*	*0.16*	*0.31*	*0.78*	*1.55*
	pH	2.3	3.0	4.0	4.8	5.1	5.8	6.1	6.7	8.2
Ignacio, Soft Limed, good	*cc.*	*2.15*	*1.08*	*0.43*	*0.22*	*0.00*	*0.11*	*0.23*	*0.55*	*1.10*
	pH	3.0	4.3	5.2	6.3	6.9	7.4	7.7	8.0	8.4

The nutrient salts of the soil are of very little importance as buffers as can be seen from table 8 where all "B" soils are unplanted and therefore rich in salts, and the "A" soils have been cropped for six years and are therefore rather exhausted.[6] Liming only moves the reaction over towards the alkaline side and does not increase the buffer effect to any appreciable extent (as seen from the limed Ignacio soil).

---

[6] In table 9 the titration of a modified Hoagland's solution is given. This represents a good soil solution. It can be seen that the titratable acidity is very weak when compared with that of the soils.

It seems, considering Javanese and California garden soils as compared with the mineral soils from the same districts[7] that good humus-manuring would improve the buffer curves in a relatively short time.

Another way is suggested by comparison of the old and new soils. Through weathering and decomposition the buffer effect is increased. This process can be accelerated by cultivation.

It seems as if it would be possible to ascertain the lime requirement of a soil by titration. By lime requirement, one means two things, partly the amount of lime required for "neutralizing" a soil and partly, in lime-poor soils, the amount required to fill the nutrition balance. The "neutralizing" lime only, is to be considered here.

TABLE 9

Effect of addition of acid or alkali upon the hydrogen-ion concentration of modified Hoagland's solution

	0 1 *N* HCl		0 1 *N* NaOH					
Cc. of acid or alkali added per gm. solution....	0 03	0 01	0 00	0 08	0 22	0 57	0 77	0 88
Resulting reaction of modified Hoagland's solution.	3	4	5	6	7	8	9	10

A review of the literature shows that many methods have been used to determine soil acidity. There are three different types of these methods. First, the titration method in which only one actual pH determination is made, namely, the end point of the titration. Some use phenolphthalein, and others, other indicators.

Another type is that in which only a qualitative test with an arbitrarily chosen method is made.

Christensen used a biological method in which the growth of Azotobacter was the indicator.

What is done in all these cases and what it is aimed to do is to change the reaction of the soil to a certain point. Why not do it by titration with a strong but diluted acid (or alkali) in order to get the reaction as quickly as possible and then locate the different points on the pH scale. By using this method the arbitrariness would be reduced to a minimum and one would obtain a universal and simple method for the determination of the lime requirement.

SUMMARY

The potential acidity of the soil or the buffer action is an important and nearly overlooked factor. It has been found that in some cases there is a good correlation between the type of titration curve and fertility. A strong buffer action means a good soil, a weak one a bad or easily changed soil, provided the reaction of the soil lies within certain limits. Humus-manuring and cultivation are the two factors for increasing the buffer effect.

---

[7] A good suggestion in this work would be to select a certain green manure crop of which the manure is as near neutral as possible in order to avoid too much liming.

The titration of soils may be used for determination of the lime requirement.

REFERENCES

(1) Arrhenius, O. 1921 The hydrogen-ion concentration of Egyptian soils and the reclamation of alkali land. *In* Jour. Cairo. Sci. Soc. v. 10, no. 104–105.

(2) Arrhenius, O. 1921 The influence of soil reaction on earthworms. In Ecology, v. 2, p. 255–257.

(3) Arrhenius, O. 1922 A factor influencing the growth of the wilt disease. Deli Proofstation, Sumatra. Mededel.

(4) Arrhenius, O. 1922 A possible correlation between the fertility of rice soils and their titration curves. *In* Soil Sci., v. 14, p. 21–26.

(5) Bjerrum, N., and Gjaldbaek, J. K. 1919 Undersögelser over de faktorer som bestemme jordbundens reaktion (Investigations on the reaction-determining factors of the soil). *In* Veterinaer o. Landbohösk. Aarskrift. (German resume), p. 48–91.

(6) Christensen, H. R. 1916 Undersögelser over Fremgangsmaader til bestemmelse af jordens reaktion. Tidsskr. Planteavl, 231–83.

(7) Fred, C. B., and Davenport, A. 1918 Influence of reaction on nitrogen assimilating bacteria. *In* Jour. Agr. Res. 14, 317–336.

(8) Gillespie, L. J. 1920 Colorimetric determination of hydrogen-ion concentration without buffer mixtures with special reference to soils. *In* Soil Sci., v. 9, no. 2, p. 115–136.

(9) Hildebrand, J. H. 1913 Some applications of the hydrogen electrode in analysis, research and teaching. *In* Jour. Am. Chem. Soc., v. 35, p. 847–871.

(10) Hoagland, D. R. 1917 The effect of hydrogen-and hydroxyl-ion concentration on the growth of barley seedlings. *In* Soil Sci., v. 3, p. 547–560.

(11) Knight, H. G. 1920 Acidity and acidimetry of soils. IV. Proposed method for determinations of lime requirement of soils. *In* Jour. Ind. Eng. Chem., v. 12, p. 559–562.

(12) Miyake, K. 1916 Toxic action of soluble aluminum slats upon the growth of rice plant. *In* Jour. Biol. Biol. Chem., v. 25, p. 23–28.

(13) Salter, R. M., and McIlvaine, T. C. 1920 Effect of reaction of solution on germination of seed and on growth of seedlings. *In* Jour. Agr. Res., v. 19, p. 73–96.

(14) Sharp, L. T., and Hoagland, D. R. 1916 Acidity and absorption in soils as measured by the hydrogen-ion electrode. *In* Jour. Agr. Res. v. 7, p. 124.

(15) Stewart, G. R. 1918 Effect of season and crop growth in modifying the soil extract. *In* Jour. Agr. Res., v. 12, p. 311–360.

EFFECT OF CERTAIN CALCIUM COMPOUNDS AND OTHER SUBSTANCES ON THE YIELD AND CALCIUM CONTENT OF SOME CROPS[1]

O. M. SHEDD[2]

Kentucky Agricultural Experiment Station

Received for publication October 14, 1921

HISTORICAL

In a former publication (7), the writer has shown by an improved method of analysis that many Kentucky soils have a very low total calcium content; in fact, from work done on several of the same samples, some of which has been published (6, p. 267–306) the calcium deficiency in many of our soils assumes equal importance with their low phosphorus and nitrogen content when the relative amounts of these elements removed by ordinary crops are considered.

At present, enormous quantities of limestone are being used on our soils to increase production. Very little significance has been attached, however, to the fact that in applying this material an essential element for plant growth is added and one which is removed in comparatively large quantities by crops.

It has been assumed heretofore that soils generally contain abundant calcium compounds to furnish an ample supply of this element for crop requirements. As soil-survey work has progressed, however, the accumulated data show that there are certain types in which the small percentage of total calcium found would indicate that there may be a deficiency of this element for permanent fertility. As a result, recent investigators and agricultural writers, for example, William Frear,[3] Halligan (2, p.21), Hopkins (3, p. 38 and succeeding reports), Thorne (8, p. 57), Van Slyke (9, p. 21), and Voorhees (10, p.3) emphasize its importance and class it as one of the four probable limiting elements the others being nitrogen, phosphorus, and potassium. The assumption that calcium is of minor importance in most soils probably accounts for the fact that very few experiments have been carried on to determine its effect as a plant food element.

McIntire and Willis (4) described some experiments in which a comparison was made of the carbonates and silicates of calcium and magnesium on the growth of red clover in pot experiments, employing two types of soil. They obtained better results with calcium silicate than with the carbonate. In their experiments, equal amounts of calcium were compared in the two materials, the silicate used being the native mineral, wollastonite. These authors also quote experiments made by Mieth (5) in which this investigator concludes that the easily decomposable calcium silicate should receive consideration as a source of lime for growing plants.

---

[1] Published by permission of the Director of the Kentucky Agricultural Experiment Station.

[2] The writer desires to express his appreciation of the helpful suggestions offered by Dr. A. M. Peter, Head of the Department of Chemistry, during the progress of the work.

[3] In a personal letter to the author.

EXPERIMENTAL WORK

In view of the fact that many of our soils have been found to have a relatively low total calcium content, the experiments below were intended to determine, if possible, the effect of this element as plant food on the growth of certain plants in such soils.

It is very difficult to test the influence of calcium as an essential element of plants by adding a compound to the soil in which they are to be grown, because this brings into the problem the effect of the other elements contained in the same directly upon the plant as well as the effect of the compound upon certain properties of the soil, such as acidity and texture which influence plant growth. The plan adopted for these experiments was to compare the effect of adding calcium silicate, calcium citrate, or calcium oxalate, respectively, with that of adding magnesium silicate, silica or dextrin, carrying equivalent amounts of magnesium, silicon, or carbon.

The principal comparison was that of the calcium and magnesium silicates in equivalent amounts. It was assumed that the secondary effect of the latter compound upon the acidity of the soil would about equal that of the former, giving opportunity for the calcium to show its effect as plant food. The use of magnesium silicate to check the neutralizing effect of the calcium silicate was considered preferable to the use of either calcium or magnesium carbonate.

The silica treatments were carried on as a control on the silica contained in the calcium silicate, consequently the amount used exceeds that contained in the magnesium silicate. It may therefore be used to check both. The dextrin was used as a control on the carbon added in the calcium citrate and, incidentally, that in the calcium oxalate. The quantity of carbon added in the dextrin applied therefore equals that of the former and is about double that of the latter compound.

The materials were stock precipitated chemicals which in most instances were further purified by washing with water. Analyses are given in table 1.

The soils used in the experiments were taken from areas which showed a low average calcium content. They do not, however, represent the soils with the lowest calcium content to be found in these areas, but, on the contrary, some are above the average. Partial analyses of the soils used and other data follow:

TABLE 1

Percentage composition of chemicals used in pot experiments

	CALCIUM SILICATE	MAGNESIUM SILICATE	SILICA	CALCIUM CITRATE	CALCIUM OXALATE	DEXTRIN
Ignition	10.82	23.20*	4.58	69.98	61.38	99.84
SiO_2	63.22	60.68	93.26		0.06	0.16†
NH_3ppt	0.52	0.86				
CaO	17.70	0.70		29.76	38.80	0.02
MgO	0.43	13.11			0.11	
Na_2O	7.70		1.26		0.59	
K_2O	0.09		0.06		0.03	
Mn_3O_4					0.10	
Approximate formula‡	$2CaO, Na_2O, 7SiO_2, 4H_2O$	$MgO, 3SiO_2, H_2O$		$Ca_3C_{12}H_{10}O_{14}, 4H_2O$	CaC_2O_4, H_2O	

* Moisture.

† Ash.

‡ Calculated from analysis.

Soil no. 56583, Bath County, in the Devonian area. Surface soil from the farm of W. W. Penix, about 1½ miles southwest of Olympia, Ky.

	TOTAL	SOLUBLE IN 0.2 N HNO_3	SOLUBLE IN CARBONATED WATER
		pounds in 2,000,000	
Phosphorus	1180	86	
Calcium	4473	1080	700
Limestone requirement	5828		

Soil no. 56584, Russell County, in the Keokuk-Waverly area. Surface soil from the farm of J. S. Dickenson, about 5 miles southwest of Dunnville, Ky., on the Dunnville and Jamestown Pike. Commercial fertilizers have been used.

	TOTAL	SOLUBLE IN 0.2 N HNO_3	SOLUBLE IN CARBONATED WATER
		pounds in 2,000,000	
Phosphorus	440	32	
Calcium	2720	1160	380
Limestone requirement	100		

Soil no. 56585, Floyd County, in the Eastern Coal Field area. Surface soil from the farm of Burr Hereford, near Cliff, Ky.

	TOTAL	SOLUBLE IN 0.2 N HNO_3	SOLUBLE IN CARBONATED WATER
		pounds in 2,000,000	
Phosphorus	1580	36	
Calcium	1420	1580	560
Limestone requirement	2366		

Soil no. 56586, Laurel County, in the Eastern Coal Field area. Surface soil from the control plots of the Station experiment field near Fariston, Ky.

	TOTAL	SOLUBLE IN 0.2 N HNO_3	SOLUBLE IN CARBONATED WATER
		pounds in 2,000,000	
Phosphorus	1100	24	
Calcium	3500	1260	320
Limestone requirement	774		

Soil no. 56587, Hardin County, in the St. Louis-Chester area. Surface soil from the farm of Harry Gatton, 3 miles northwest of Glendale, on the Bacon Creek Pike. Commercial fertilizers have been used.

	TOTAL	SOLUBLE IN 0.2 N HNO_3	SOLUBLE IN CARBONATED WATER
		pounds in 2,000,000	
Phosphorus	820	26	
Calcium	3140	1240	220
Limestone requirement	414		

Soil no. 56588, Taylor County, in the St. Louis-Chester area. Surface soil from the farm of S. B. Coppock, 1 mile south of Burdick, Ky.

	TOTAL	SOLUBLE IN 0 2 N HNO_3	SOLUBLE IN CARBONATED WATER
		pounds in 2,000,000	
Phosphorus	780	38	
Calcium	3580	2040	480
Limestone requirement	107		

Each soil was thoroughly mixed, put through a coarse sieve, and air-dried. The experiments were carried on in the greenhouse in 2-gallon stone jars supplied with drainage and 15 pounds of soil was used in each jar. Triplicate treatments were given in all instances, and equal amounts of distilled water were applied.

In the experiments below it is assumed that an acre of the surface soil, 6 inches in depth, weights 2,000,000 pounds. The treatments given were as follows:

Controls. Di-potassium phosphate was added to these and other pots of the series as described later.

Magnesium silicate. An amount carrying 607 pounds magnesium and 2186 pounds silicon per 2,000,000 pounds.

Calcium silicate. An amount carrying 1000 pounds calcium and 2346 pounds silicon per 2,000,000 pounds.

Silica. An amount carrying 2346 pounds silicon per 2,000,000 pounds.

Calcium citrate. An amount carrying 1000 pounds calcium and 1198 pounds carbon per 2,000,000 pounds.

Calcium oxalate. An amount carrying 1000 pounds calcium and 598 pounds carbon per 2,000,000 pounds.

Dextrin. An amount carrying 1198 pounds carbon per 2,000,000 pounds.

Each of these chemicals was put through 100-mesh sieve and thoroughly mixed with the soil in each instance. An application of a solution of di-potassium phosphate at the rate of 100 parts per 2,000,000 of soil was made to each pot at the beginning of the experiment.

The crops grown in rotation were soybeans, sweet clover, alfalfa, and oats. The soils were inoculated before planting each legume, and between crops they were stirred to a depth of 6 inches and pulverized. The plants were thinned to uniform size and distribution, and the number allowed to each pot was 5 for the soybeans, sweet clover, and alfalfa and 10 for the oats. Repeated cuttings of the sweet clover and alfalfa near the crown were made.

At harvest, the plants were cut close to the ground, the roots disregarded, and the air-dried weights were obtained. Composite samples were made of the plants and separately of the seed obtained from the triplicate pots in each treatment for the laboratory work. The samples were finely ground, after which ash and calcium determinations were made on them. The calcium was determined volumetrically by practically the same procedure followed in the improved method on soils mentioned above (7).

In order to test the effect of the silicates and other treatments on the lime requirement of the soils, acidity determinations by the Hopkins method (1) were made before and after the materials were applied and after the crops had been grown. These results together with the weights of the crops from the three pots of each treatment and other data are given in tables 2–12.

TABLE 2

Yield of soybeans (air-dry)

TREATMENT	BATH COUNTY		RUSSELL COUNTY		FLOYD COUNTY		LAUREL COUNTY		HARDIN COUNTY		TAYLOR COUNTY	
	Hay	Seed	Hay	Seed	Hay	Seed	Hay	Seed	Hay	Seed	Hay	Seed
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Control	19.76	2.07	19.00	2.45	16.75	0.53	26.03	0.89	27.16	1.53	17.89	1.45
Magnesium silicate	20.18	2.38	20.15	3.63	15.28	2.19	28.53	0.76	33.23	0.87	20.97	0.27
Calcium silicate	22.02	3.45	22.34	1.92	20.32	0.85	32.92	0.40	33.34	0.18	25.75	0.52
Silica	17.94	1.79	18.16	3.15	18.60	0.87	27.64	0.67	28.17	0.63	25.35	0.29
Calcium citrate	18.28	2.44	18.77	1.51	15.69	1.38	23.26	2.04	25.91	1.34	24.65	0.75
Calcium oxalate	19.54	1.55	20.13	2.84	17.44	1.19	30.34	1.19	30.11	1.15	25.52	1.33
Dextrin	19.84	0.74	15.51	0.97	11.29	1.14	24.02	1.27	18.22	1.74	20.18	1.51

TABLE 3

Yield of sweet clover and alfalfa (air-dry)

TREATMENT	SWEET CLOVER			ALFALFA		
	Bath County	Russell County	Floyd County	Laurel County	Hardin County	Taylor County
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Control	(No growth obtained)	17.27	9.53	19.85	20.69	21.12
Magnesium silicate	4.77	34.44	16.17	28.38	32.90	23.58
Calcium silicate	13.48	36.39	18.83	32.56	44.22	32.64
Silica	(No growth obtained)	13.14	8.44	13.00	20.83	22.30
Calcium citrate	8.79	35.75	16.36	28.64	35.04	30.08
Calcium oxalate	11.74	29.94	12.82	31.20	32.25	30.64
Dextrin	(No growth obtained)	16.08	8.14	19.73	24.33	18.93

DISCUSSION OF RESULTS

The yields shows that some of the soils responded favorably to the application of calcium compounds, but the increases obtained were more pronounced with some crops and treatments than with others, as shown below.

Soybeans. The highest yield of hay on each soil was obtained by the use of calcium silicate, but on only one soil did this treatment give the highest yield of seed, the remainder being lower than some of the controls comprising the magnesium silicate, silica, and control plots. The calcium citrate showed an increase in hay on one and in seed on three soils, while calcium oxalate gave increases in hay on five and in seed on two soils, compared with the dextrin and control soils. The yields of seed are so small and irregular in some instances that they are insignificant.

Sweet clover and alfalfa. All calcium treatments showed material gains on all soils. The results obtained with sweet clover on the Bath County soil are

TABLE 4

Yield of oats (air-dry)

TREATMENT	BATH COUNTY		RUSSELL COUNTY		FLOYD COUNTY		LAUREL COUNTY		HARDIN COUNTY		TAYLOR COUNTY	
	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Control	49.79	13.79	30.98	10.32	27.55	8.44	25.83	7.55	26.79	8.56	28.17	7.50
Magnesium silicate	40.58	7.17	47.41	14.97	38.04	10.29	29.88	8.63	41.61	13.64	52.10	12.86
Calcium silicate	40.19	11.95	55.43	19.41	35.98	10.42	31.18	10.44	47.96	21.56	41.69	14.28
Silica	44.98	16.03	30.29	9.66	30.43	10.09	27.48	8.65	26.35	9.21	25.58	6.86
Calcium citrate	24.78	6.19	40.67	13.04	35.35	11.23	32.59	12.22	41.92	15.80	38.24	10.84
Calcium oxalate	26.01	5.93	39.23	15.30	26.19	7.28	30.82	11.72	31.97	12.16	35.18	11.46
Dextrin	44.65	15.24	30.50	10.49	28.93	9.42	29.59	9.56	28.11	10.81	34.34	10.43

of interest since no growth was obtained in three of the four control treatments. Incidentally this soil showed the highest acidity. With some treatments the growth of sweet clover was nearly inversely proportional to the acidity found, but this was not always true.

Oats. Calcium silicate made gains in the straw on three and in the grain on five soils. Calcium citrate shows gains in both grain and straw on five soils and calcium oxalate on four soils, compared with the controls.

The good results obtained with magnesium silicate are interesting and cannot be entirely attributed to the neutralization of the acidity by this material. It is thought that part of the beneficial effect is due to the magnesium, as illustrated in some of the results obtained on the yield of seed. In this connection it might be mentioned that plant physiologists are agreed that magnesium bears some close relation to seed formation. Considering the amount applied, the fact that this material did not prove detrimental to growth is of interest since the calcium-magnesium ratio in the soils was materially changed by its addition. The beneficial effect obtained from the use of the magnesium silicate raises the question of possible deficiency of magnesium in soils, and it is the writer's intention to investigate this more fully.

TABLE 5

Ash content of soybean hay (air-dry)

TREATMENT	BATH COUNTY	RUSSELL COUNTY	FLOYD COUNTY	LAUREL COUNTY	HARDIN COUNTY	TAYLOR COUNTY	AVERAGE
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Control	6.15	6.07	5.65	7.32	10.76	10.84	7.80
Magnesium silicate	7.37	6.94	6.42	8.45	8.96	11.39	8.26
Calcium silicate	8.02	7.54	6.74	8.99	9.62	10.59	8.58
Silica	6.97	6.96	5.47	7.45	9.55	10.52	7.82
Calcium citrate	6.70	6.57	5.67	6.91	9.73	10.20	7.63
Calcium oxalate	7.25	6.42	6.07	7.43	11.21	8.38	7.79
Dextrin	6.52	6.95	5.88	8.28	9.81	12.42	8.31

TABLE 6

Ash content of sweet clover and alfalfa hay (air-dry)

TREATMENT	SWEET CLOVER				ALFALFA			
	Bath County	Russell County	Floyd County	Average	Laurel County	Hardin County	Taylor County	Average
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Control	*	7.80	10.09	8.95	7.20	8.38	6.86	7.48
Magnesium silicate	8.16	6.29	8.68	7.71	6.60	7.31	7.60	7.17
Calcium silicate	9.76	7.29	8.27	8.44	6.43	6.96	7.19	6.86
Silica	*	8.87	8.11	8.49	8.25	9.11	7.05	8.14
Calcium citrate	10.30	6.88	8.94	8.71	7.67	6.89	7.13	7.23
Calcium oxalate	8.92	6.89	11.41	9.07	7.10	8.37	6.42	7.30
Dextrin	*	8.13	8.51	8.32	8.02	7.81	7.76	7.86

* No growth obtained.

TABLE 7

Ash content of oats (air-dry)

TREATMENT	BATH COUNTY		RUSSELL COUNTY		FLOYD COUNTY		LAUREL COUNTY		HARDIN COUNTY		TAYLOR COUNTY		AVERAGE	
	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain
	per cent		*per cent*		*per cent*		*per cent*		*per cent*		*per cent*		*per cent*	
Control	5.40	3.10	4.87	2.82	7.33	3.66	5.62	3.30	6.16	3.46	6.72	3.79	6.02	3.36
Magnesium silicate	9.17	4.39	7.81	4.37	11.62	4.41	13.23	4.71	9.48	4.47	9.63	4.78	10.16	4.52
Calcium silicate	7.16	4.60	8.44	4.66	10.64	4.56	10.88	4.47	6.86	4.31	10.26	4.67	9.04	4.55
Silica	4.93	3.14	5.49	3.20	6.46	3.94	6.88	3.32	6.76	3.60	8.09	4.06	6.44	3.54
Calcium citrate	7.05	3.39	4.12	2.40	6.28	3.19	5.19	3.10	5.42	2.88	6.61	3.29	5.78	3.04
Calcium oxalate	7.16	3.85	4.06	2.30	7.07	3.61	5.44	2.97	5.86	3.28	5.93	3.22	5.92	3.21
Dextrin	5.79	3.09	5.52	2.73	6.26	3.45	5.46	3.04	6.89	2.94	6.29	3.27	6.04	3.09

TABLE 8

Calcium content of soybeans (air-dry)

TREATMENT	BATH COUNTY		RUSSELL COUNTY		FLOYD COUNTY		LAUREL COUNTY		HARDIN COUNTY		TAYLOR COUNTY		AVERAGE	
	Hay	Seed	Hay	Seed	Hay	Seed	Hay	Seed	Hay	Seed	Hay	Seed	Hay	Seed
	per cent		*per cent*		*per cent*		*per cent*		*per cent*		*per cent*		*per cent*	
Control	1.16	0.141	1.82	0.135	1.50	Lost	2.30	0.179	1.99	0.157	2.35	0.169	1.85	0.156
Magnesium silicate	1.17	0.121	1.34	0.115	1.54	0.102	2.06	0.170	1.78	0.129	1.87	0.337	1.63	0.162
Calcium silicate	2.05	0.106	2.31	0.108	2.14	0.012	2.89	0.160	2.55	0.213	2.77	0.111	2.45	0.118
Silica	1.23	0.124	1.90	0.116	1.54	0.129	2.33	0.196	2.08	0.305	2.33	0.308	1.90	0.196
Calcium citrate	1.68	0.101	2.09	0.129	1.80	0.012	2.53	0.160	2.38	0.167	2.32	0.124	2.13	0.116
Calcium oxalate	1.76	0.124	2.30	0.093	1.96	0.080	2.72	0.158	2.26	0.158	2.45	0.125	2.24	0.123
Dextrin	1.00	0.144	1.97	0.154	1.48	0.021	2.43	0.159	2.01	0.181	2.29	0.221	1.86	0.147

Calcium silicate increased the average ash content of the soybean hay and oat grain, but neither this nor the other calcium treatments increased the ash of the other crops. Calcium silicate also increased the average percentage of calcium in the soybean hay, and the oat grain and the other calcium treatments increased the amount of this element in the soybean hay and in both the oat straw and grain. Calcium oxalate seems to have raised the calcium content of the alfalfa.

It is of interest to observe in table 12 that the relative acidities of the six soils remain the same after each treatment, the order beginning with the lowest acidity being Russell, Taylor, Hardin, Laurel, Floyd, and Bath. The only deviations from this order are in the silica series where Russell is larger than Taylor and in the calcium oxalate series where Hardin and Laurel test equal in acidity. The effect of the different treatments, however, is much more pronounced in some soils than in others, and it is difficult to explain the behavior of the same material in its influence on the acidity of different soils.

TABLE 9

Calcium content of sweet clover and alfalfa (air-dry)

TREATMENT	BATH COUNTY	RUSSELL COUNTY	FLOYD COUNTY	AVERAGE	LAUREL COUNTY	HARDIN COUNTY	TAYLOR COUNTY	AVERAGE
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Control	*	2.08	1.98	2.03	1.65	1.46	1.62	1.58
Magnesium silicate	1.06	1.17	1.35	1.19	1.03	1.01	1.40	1.15
Calcium silicate	1.52	1.84	1.79	1.72	1.39	1.28	1.43	1.37
Silica	*	2.44	1.72	2.08	1.55	1.38	1.57	1.50
Calcium citrate	1.83	2.02	1.94	1.93	1.52	1.49	1.75	1.59
Calcium oxalate	1.67	2.05	2.42	2.05	1.58	1.66	1.84	1.69
Dextrin	*	2.22	1.38	1.80	1.48	1.33	1.82	1.54

* No growth obtained.

When the results of the pot tests are considered, there is some evidence which shows that the calcium in the different treatments has probably functioned as plant food in some instances and, furthermore, has exerted an influence on the ash and calcium content of the plant. Comparison of the averages of the calcium treatments with those of the no-calcium treatments in table 11 shows that more of this element was removed by the crops from the former than by that from the latter.

If there had been an adequate supply of available calcium present in the soil it would seem that some of these differences should not be so large especially where magnesium silicate was applied, since this substance materially reduced the acidity of the soil.

Many of our soils have a lower total calcium content than those used in these experiments. Taking into consideration that an application of 1 ton of limestone or of calcium phosphate per acre to some of our poor soils adds about as much calcium as is already present, there can hardly be any doubt that any in-

TABLE 10

Calcium content of oats (air-dry)

TREATMENT	BATH COUNTY		RUSSELL COUNTY		FLOYD COUNTY		LAUREL COUNTY		HARDIN COUNTY		TAYLOR COUNTY		AVERAGE	
	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain
	per cent		*per cent*		*per cent*		*per cent*		*per cent*		*per cent*		*per cent*	
Control	0.374	0.086	0.564	0.105	0.423	0.096	0.497	0.152	0.450	0.115	0.455	0.117	0.461	0.112
Magnesium silicate	0.302	0.074	0.386	0.089	0.338	0.090	0.372	0.113	0.363	0.103	0.355	0.117	0.353	0.098
Calcium silicate	0.349	0.094	0.469	0.146	0.507	0.107	0.546	0.128	0.396	0.126	0.405	0.129	0.445	0.122
Silica	0.355	0.105	0.514	0.115	0.388	0.083	0.458	0.110	0.397	0.103	0.497	0.126	0.435	0.107
Calcium citrate	0.470	0.097	0.795	0.139	0.490	0.114	0.649	0.150	0.511	0.116	0.661	0.140	0.596	0.126
Calcium oxalate	0.511	0.133	0.802	0.153	0.631	0.123	0.757	0.145	0.679	0.134	0.590	0.116	0.662	0.134
Dextrin	0.412	0.104	0.659	0.119	0.414	0.094	0.410	0.117	0.461	0.077	0.401	0.104	0.460	0.103

TABLE 11

Calcium removed by crops; average for each group of three pots for each crop on all soils

TREATMENT	SOYBEANS, HAY AND SEED	SWEET CLOVER	ALFALFA	OATS, STRAW AND GRAIN
	gm.	*gm.*	*gm.*	*gm.*
Control	0.393	0.181	0.325	0.156
Magnesium silicate	0.379	0.220	0.325	0.158
Calcium silicate	0.641	0.394	0.500	0.205
Silica	0.433	0.150	0.281	0.145
Calcium citrate	0.451	0.392	0.497	0.227
Calcium oxalate	0.536	0.372	0.530	0.223
Dextrin	0.340	0.145	0.323	0.162
Average of calcium treatments	0.543	0.386	0.509	0.218
Average of no calcium treatments	0.386	0.174	0.314	0.155
Difference	0.157	0.212	0.195	0.063

TABLE 12

Effect of treatments and growth of plants on lime requirement

TREATMENT	BATH COUNTY		RUSSELL COUNTY		FLOYD COUNTY		LAUREL COUNTY		HARDIN COUNTY		TAYLOR COUNTY	
	Lime requirement‡	Relative acidity§	Lime requirement	Relative acidity	Lime requirement	Relative acidity	Lime requirement	Relative acidity	Lime requirement	Relative acidity	Lime requirement	Relative acidity
	lbs.		*lbs.*		*lbs.*		*lbs.*		*lbs.*		*lbs.*	
Control*	5828	100	100	100	2366	100	774	100	414	100	107	100
Control†	6281	108	71	71	1413	60	232	30	125	30	54	50
Magnesium silicate	4561	78	18	18	535	23	89	11	61	15	54	50
Calcium silicate	3690	63	11	11	107	5	46	6	39	9	36	34
Silica	5064	87	61	61	964	41	253	33	132	32	43	40
Calcium citrate	3073	53	18	18	221	9	57	7	54	13	29	27
Calcium oxalate	2884	49	18	18	111	5	43	6	43	10	25	23
Dextrin	4896	84	71	71	1453	61	278	36	143	35	64	60

* Soil used in experiments before any plants were grown and to which nothing has been added. Tested Dec. 7, 1918.

† Same soil after addition of 100 parts K_2HPO_4 per 2,000,000 and all crops were grown. Tested Aug. 28, 1920.

‡ Calcium carbonate required per acre to neutralize acidity.

§ Original soil = 100.

creased growth obtained as a result of applying these materials, or even of some commerical fertilizers to such soils, is due in part at least to the plant food calcium which they supply in addition to the other good effects which they may accomplish.

SUMMARY

1. Pot experiments in the greenhouse were made on six soils representing four different types in this State in an effort to determine if there was a deficiency of plant-food calcium.

2. Following applications of different calcium salts, four crops—namely, soybeans, sweet clover, alfalfa and oats—were grown in rotation. The effects of certain other substances were studied at the same time as a means of control.

3. In some instances the addition of calcium compounds to these soils increased the yield of some crops, both in grain and straw or hay.

4. The calcium treatments increased the ash and calcium content of some of the crops.

5. A considerable variation in the percentages of ash and calcium of the same plant, both in grain and straw or hay, was found when grown on different soils or even on the same soil where different treatments were given.

6. Silica treatments appear to have exerted a favorable influence on the yield of soybeans and oats on some soils.

7. Appreciable gains were obtained by the use of magnesium silicate in many instances, especially on the yield of some grain. These may be attributed in part at least to the beneficial affects of the magnesium, although there was some reduction of the soil acidity by this treatment.

8. Where magnesium silicate was applied to the soil the average calcium content of the hay or straw of all crops grown and of the grain of oats was lower than that by any other treatment.

9. The acidity of the soils was materially reduced by some treatments, but it is not believed that all of the increased yields can be entirely attributed to this fact. The effect of the same treatment on the acidity of different soils produced some interesting results which are difficult to explain.

10. Comparison of the averages of calcium with no-calcium treatments shows that the crops of the former usually removed much larger quantities of this element from the soil. This was probably due to the fact that where no calcium threatment was given, the plants were in need of an adequate supply available calcium for growth.

REFERENCES

(1) ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS 1908 Official and provisional methods of analysis. U. S. Dept. Agr., Bur. Chem. Bul. 107 (revised).

(2) HALLIGAN, JAMES EDWARD 1912 Soil Fertility and Fertilizers, p. 21. Chemical Publishing Company, Easton, Pa.

(3) HOPKINS, C. G., ET AL. 1911 Moultrie County soils. Ill. Agr. Exp. Sta. Soil Rpt. 2.

(4) MACINTIRE, W. H., AND WILLIS, L. G. 1914 Comparison of silicates and carbonates as sources of lime and magnesia for plants. *In* Jour. Ind. and Eng. Chem., v. 6, no. 12, p. 1005–1008.

(5) MIETH, HANS 1910 Ist der Kalk des kieselsauren Kalkes zur Ernahrung der Pflanze geeignet? *In* Landw. Vers. Stat., vol. 74, no. 1/2, p. 81–120.

(6) SHEDD, O. M. 1913 The sulfur content of some typical Kentucky soils. Ky. Agr. Exp. Sta. Bul. 174.

(7) SHEDD, O. M. 1920 A proposed method for the estimation of total calcium in soils and the significance of this element in soil fertility. *In* Soil Sci., v. 10, no. 1, p. 1–14.

(8) THORNE, CHARLES E. 1914 Farm manures, p. 57. Orange Judd Company, New York.

(9) VAN SLYKE, LUCIUS L. 1912 Fertilizers and Crops, p. 21. Orange Judd Company, New York.

(10) VOORHEES, EDWARD B. 1903 Fertilizers, 5th ed., p. 3. Macmillan Company, New York.

INFLUENCE OF SULFUR OXIDATION UPON GROWTH OF SOY BEANS AND ITS EFFECT ON BACTERIAL FLORA OF SOIL[1]

W. RUDOLFS[2]

New Jersey Agricultural Experiment Stations

Received for publication October 18, 1921

The oxidation of sulfur in the soil, and the influence of sulfur upon bacterial activities and development has been studied by many investigators. Sulfur transformation in the soil has long been known, but only in recent years has the mechanism of it been more fully under consideration. Although no attempt is made to give a complete bibliography on the subject, some of the more conclusive studies reported are included in the following review of literature.

Boulanger and Dugardin (3), in trying to explain the fertilizing action of sulfur, found that the effect of sulfur on crop yields was more marked with unsterilized soil than with sterilized soil, due to the oxidation of sulfur by bacterial activities. Demolon (6) studying the fertilizer action of sulfur found that but little sulfur was oxidized in a sterilized garden soil, and reached the same conclusions. Bernhard (2) states that the beneficial effect of sulfur for the control of potato scab, was due to the disinfection of the soil by sulfur. Brown and Kellogg (5) showed that different soils have unlike "sulfofying powers" and some of the factors influencing the change of elemental sulfur to the sulfate form were of a biological nature. Lipman and his associates (11) have shown that elemental sulfur is oxidized by the proper bacteria. Later studies at the New Jersey Experiment Stations, have confirmed the earlier experiments. Martin (13) recently published results obtained with inoculated and uninoculated sulfur for the control of potato scab, which show that inoculated sulfur produced greater amounts of soil acidity then did uninoculated sulfur, and was superior for the control of potato scab.

There has been, and still is, considerable controversy as to the necessity of applying sulfur to the soil for the stimulation of plant growth. Lyon and Bizzel (12) report that in the lysimeter experiments at Cornell the sulfate sulfur in the drainage water was from three to six times as great as in the crops, while the sulfur content of the drainage water from the unplanted soil was about equal to the sulfur content of the crops and drainage water from the planted soil. Swanson and Miller (21) state that the loss of sulfur due to the amount taken up by crop is insignificant as compared with the total amount which has disappeared from the soil. Hart and Peterson (8) calculate the loss of sulfur in drainage water to be three times the amount brought down to an acre from the atmosphere. Stewart (20) concludes that under humid conditions sulfur need not be added to the soil as plant food. Sufficient sulfur is brought down by the rainfall to supply plant needs. Ames and Boltz (1) conclude that the cultivation of silt loam for 16 years without the addition of fertilizers has decreased the total sulfur supply.

---

[1] Paper No. 93 of the Journal Series, New Jersey Agricultural Experiment Stations, Department of Soil Chemistry and Bacteriology.

This paper will appear in RUTGERS COLLEGE STUDIES, vol. 1.

[2] Part of a thesis submitted to the faculty of Rutgers College and the State University of New Jersey in partial fulfillment of the requirements for the degree of doctor of philosophy, May 13, 1921.

Some striking results have been obtained with the use of sulfur as a fertilizer or stimulant. Reimer (17) obtained large increases in alfalfa yields by the use of elemental sulfur. A number of Oregon soils are deficient in sulfur and respond readily to sulfur applications as is shown by the work of Reimer and Tartar (18), who report increases of from 50 to 1000 per cent for alfalfa and clover by the use of fertilizers containing sulfur on various soil types. Flowers of sulfur produced beneficial results similar to those obtained by the use of superphosphate, gypsum, iron sulfate, etc. Headley (9, p. 16–18) reports experiments with sulfuric acid and gypsum and states that the yields of the soils so treated has been decidedly greater each year than those from the untreated plots, but not enough greater to be profitable. Miller (14) concludes from pot experiments that the addition of elemental sulfur enhanced the growth of the plants, and that the corresponding increases obtained on the soil extract indicated that sulfur acted directly in promoting this growth. Tottingham and Hart (23) made greenhouse and field tests with sulfur and composted sulfur and rock phosphate. They conclude that on two loam soils sulfur increased the growth of clover and cruciferae on one soil and not on the other. Sulfur increased the yields of barley seed on a silt loam apparently in need of lime. One hundred pounds of sulfur per acre was most effective.

The action of sulfur and the sulfates formed in the soil upon the soil bacteria and their activities has been studied in several instances. Janicaud (10) states that sulfur has a favorable influence on the development of bacteria in the soil. Duley (7) concludes that sulfur and gypsum increase the number of nodules to a marked extent on red clover roots in certain Missouri soils. Boulanger and Dugardin (3) studied the effect of sulfur on ammonification, nitrification, and nitrogen fixation and they found that the presence of small amounts of sulfur decidedly increased the activity of ammonifying bacteria. Brioux and Geurbet (4) challenged the conclusions of these investigators and say that sulfur as such does not have any influence upon the activities of ammonifying or nitrifying bacteria. O'Gara (15) reporting field experiments with field and truck crops in which elemental sulfur was added at a rate of 400 pounds per acre states that "one of the striking effects of sulfur treatment of soils on the soil microörganisms is the decided and consistent increase in total bacterial numbers as determined by the plate method." Tacheuchi (22) concludes that sulfur applied at the rate of 0.5 per cent and over decreases the number of bacteria in the soil, and that sulfur alone does not affect the root development, or the number of root nodules.

Miller (14) states that the great increase in the nitrogen content of clover grown on soil where sulfates had been added is the result, in all probability, of the sulfates stimulating the action of legume bacteria; the number of nodules on the clover roots were increased. Pitz (16) found elemental sulfur to increase soil acidity, and that the number of bacteria which would grow on agar plates decreased after a certain period, if sulfur was added to a silt loam soil. He reports an increase in ammonification, accompanied by a parallel decrease in nitrate formation.

EXPERIMENTS WITH SLIGHTLY ALKALINE SOILS

From the short review of literature it seems clear that some soils respond to the treatment of sulfur and others do not. The study reported below was primarily undertaken to determine whether or not a slightly alklaine soil, which produces chlorosis, would be benefited by additions of sulfur.

Determinations were made on numbers of the microörganisms in this soil, influence on the physical structure (flocculation, turbidity, water-holding capacity, change in volume), and the influence on the growth of soy beans.

The soil used was a Hanford fine sandy loam, and secured by Prof. C. F. Shaw of the University of California from ranches near Pomona, Cal. Professor Shaw wrote the following notes about these soils and their history:

> These soils were secured from a point about two miles west of the business center of Pomona. Soil 1 was taken in an orange grove about 100 feet from the west line of the orchard. The orange trees were about thirty years old. They have been manured annually with barnyard manure, but the quantity applied was unknown by the owner. He has approximately seven acres in the grove and applies to it all the manure from one horse and one cow, spreading it on as it is made. The grove has been irrigated by furrows ever since it was first set out. The water has flowed from the east towards the west and the block of soil between each row has not received water except as it has run over from the shallow furrows or has seeped in by lateral movement. There has been slight accumulation of finer sediments in the west side of the grove—the side from which the soil was taken. The soil was taken from about the middle of the row between the trees and from the low ridge between two irrigation furrows. The land had been irrigated about one week previous. These samples represent a good soil in which the fertility has been maintained reasonably well through fertilizing and through tillage.
>
> Soil 2 was taken about 150 feet west of soil 1 and about 50 feet west of the boundary line between the orange grove and the adjoining corn field. The field from which soil 2 was taken has been in corn or in barley annually for a great many years. It has been farmed nearly every year for thirty or more years, and no fertilizer has ever been applied. The ranch is run by a Mexican tenant and the system of culture is poor. During the last 6 years the field has grown at least 4 and possibly 5 crops of corn and one and possibly 2 crops of barley. At first the crop yields were good but they have dropped off during the last ten years. The field was in corn this year with a fair stand.

A mechanical analysis made of these soils showed that they were very fine. The data is given in table 1.

TABLE 1

Mechanical analyses of Hanford fine sandy loam

GRADE OF SOIL	ORANGE GROVE	OPEN FIELD
	per cent	*per cent*
Fine gravel	2.85	6.65
Coarse sand	9.99	9.34
Fine sand	11.02	11.21
Very fine sand	33.58	33.05
Silt and clay	42.56	38.91

Flowers of sulfur was inoculated with one per cent of soil which was known to contain sulfur-oxidizing organisms. This inoculated sulfur was then thoroughly mixed with soils 1 and 2 and the water-holding capacity determined. The soils were kept at 60 per cent of the water-holding capacity throughout the period of investigation.

Hydrogen-ion concentration determinations were made of the soil extract before and after mixing with sulfur and at definite intervals during the growth of the plants, using the apparatus described by Van Alstine (24).

The soils were divided into 2 parts, one to be used for plant cultures and the other for bacterial studies.

SERIES 1. VEGETATION EXPERIMENTS WITH SOIL 1

Soy beans were grown in earthenware pots filled with 5 kgm. of soil. Different amounts of inoculated sulfur were thoroughly mixed with the soil. To duplicates were added, besides the same quantities of inoculated sulfur, 300 pounds of rock phosphate per acre, and to triplicates, in the place of rock phosphate, 100 pounds of acid phosphate per acre. The exact amounts of sulfur added to all series were as follows:

CULTURE NUMBER	POUNDS PER ACRE
1, 6,11	None
2, 7,12	100
3, 8,13	300
4, 9,14	500
5,10,15	1000

The soy bean seeds were selected for size, germinated and transplanted when from 2 to 3 inches high, care being taken to select plantlets as nearly alike as possible. Six plants were planted in each pot and grown for 9 weeks. At the beginning of the experiment the soil was inoculated with a water extract of a soil containing soy-bean nodule-forming bacteria. The optimum water content of the soil was maintained by daily additions of distilled water, the pots being placed on the scale pan every 2 or 3 days. Notes were taken at intervals and the hydrogen-ion concentration of the soil extract determined once each week.

The influence of plant growth on the hydrogen-ion concentration was but slight. In the water extract of the soils treated with small amounts of sulfur the changes were not very marked, but the larger quantities of sulfur exerted a decided influence, although not as much as would be expected from the rather heavy applications. This was probably due to the slow oxidation of the sulfur.

Notes taken after 3 weeks show that, in general, the plants in soil to which sulfur was added were slightly behind the check cultures. Most of these plants started to develop small yellow spots on the leaves. According to the notes taken after 6 weeks, when 3 of the 6 plants were harvested, it appears that these yellow discolorations, which more or less resembled mosaic, were not found on the plants grown in soils which received 300, 500 and 1000 pounds of sulfur per acre, and that the plants grown in soil to which rock phosphate had also been added, had been very slightly affected, while the effect on the plants grown in the cultures receiving the same amounts of sulfur but acid phosphate instead of rock phosphate were more distinct. The yellow spots on the plants in the other cultures were at that time more pronounced than after 3 weeks. The soil used was at the beginning of the experiment slightly alkaline, or neutral, with pH values varying between 7.2 and 7.0.

A comparison made at the end of 6 weeks of all pot cultures seemed to place the plants receiving sulfur alone as best.

After 9 weeks the plants were scored again and the yellowish, sickly looking leaves counted. In all cases the yellowness increased with the increase of the quantities of sulfur employed. The plants grown in soil with sulfur alone seemed to be still ahead. At the end of 9 weeks the plants were harvested, and a comparison made on the relative numbers of nodules and on the extent of the root system.

It appeared that the check plants had all a more extensive root system than the plants grown in the sulfur-treated soils, with the exception of plants receiving 100 pounds of sulfur to the acre. On the latter the nodules were also more numerous than on the check plants and the plants treated with higher amounts of sulfur. The nodules decreased numerically with the increase of the amounts of sulfur applied. The plants which were grown in the soil receiving 1000 pounds of sulfur per acre had very few, but extremely large, nodules. Some of these nodules were ¾–1 cm. in diameter.

Although the weight of the plants decreased toward the highest sulfur application, the differences were not striking. It was concluded, therefore, to grow another crop of soy beans in the same pots with similar applications of sulfur, rock phosphate and acid phosphate.

The plants were selected and planted as before and 3 out of 6 plants harvested after 4 weeks, leaving the 3 best-looking plants in each pot. At this time the cotyledons of the check plants and of the plants receiving 1000 pounds of sulfur per acre were all still dark green, while most of the cotyledons of the plants receiving 100, 300 and 500 pounds of sulfur per acre, had been dropped previously or had turned yellow before the end of 4 weeks. The best looking plants were at that time in the check pots.

It was interesting to note that the pots receiving 1000 pounds of sulfur per acre and 300 pounds of rock phosphate in addition had a much higher hydrogen-ion concentration than the pots receiving acid phosphate in addition, and also higher than the pots which received 1000 pounds of sulfur alone. The hydrogen-ion concentration went up more or less in all cultures.

According to the notes taken, all plants were found blooming after 4½ weeks, while cultures 5, 10 and 15 had very yellow leaves of which some had been dropped at that time.

Results are given in table 2.

The results obtained were much more pronounced than those obtained with the first crop. The fact that inoculated sulfur and rock phosphate together produced poorer results than sulfur alone can not fully be explained by supposing that this particular soil does not respond to phosphorus, since the results obtained with the acid phosphate treated pot cultures indicate that some benefit might have been derived from the phosphorus treatment. The cause for the poorer results seems to lie in the greater acidity produced.

The root systems of the plants receiving 100 pounds of sulfur were, in general, more extensive than those of the plants in the check pots, but the roots of the plants in pots with higher sulfur applications were considerably less extensive.

TABLE 2

Yields of tops of second crop of soy beans grown in orange grove soil for 6 weeks

POT NUMBER	DRY WEIGHT OF TOPS	RELATIVE WEIGHT	TRANSPIRATION AND EVAPORATION	AVERAGE HEIGHT	NUMBER OF PODS	INITIAL REACTION	FINAL REACTION	REMARKS
Treated with sulfur								
	gm.		*cc.*	*cm.*		*pH*	*pH*	
1 (check)	3.0302	100.0	4150	26.5	10	6.9	6.6	
2	2.9453	97.3	4170	26.5	15	6.9	6.5	
3	2.1595	71.4	3710	21.0	2	6.6	6.3	
4	2.2741	75.1	3535	21.0	6	6.6	6.1	Slightly injured
5	2.0000	66.0	3305	21.5	5	6.2	6.0	Strongly injured, cotyledons green
Sulfur plus 300 pounds of rock phosphate								
6 (check)	3.2750	100.0	3875	21.5	13	7.0	6.7	
7	2.9348	89.6	3710	22.0	12	6.9	6.4	
8	2.8441	86.9	3755	23.0	10	6.8	6.2	
9	2.1642	66.2	3390	22.0	7	6.5	6.1	
10	1.3470	41.1	3080	19.0	0	6.1	4.6	Strongly injured, cotyledons green
Sulfur plus 100 pounds of acid phosphate								
11 (check)	3.0227	100.0	3695	24.0	8	7.0	6.7	
12	3.2712	108.1	4030	26.5	15	6.9	6.5	
13	2.5400	84.0	3480	23.0	7	6.8	6.3	
14	2.4882	82.5	3505	23.0	8	6.6	6.1	
15	1.9160	63.3	3235	21.5	3	6.2	5.8	Injured, green cotyledons

Inoculated sulfur applied in moderate quantities, therefore, might aid the plants to get hold of more plant food through stimulation of root development.

The number of nodules increased with the application of 100 pounds of sulfur but decreased considerably if greater quantities of sulfur were employed. The size of these nodules increased with the decrease of their numbers. The relative number of nodules for the different cultures, placing the checks at 100, were as follows:

CULTURE NUMBER	NODULES	CULTURE NUMBER	NODULES	CULTURE NUMBER	NODULES
1	100	6	100	11	100
2	120	7	150	12	120
3	100	8	100	13	120
4	80	9	80	14	100
5	20	10	10	15	60

The decrease in numbers of nodules for the plants receiving acid phosphate were not as great as for the plants without phosphate, and this can be attributed to the available phosphorus since the plant receiving rock phosphate decreased in numbers in the same manner as without phosphorus, and although the actual acidity of culture 5 was even much less than of culture 15, more nodules were produced in the latter. The numerical decrease of the nodules is undoubtedly due to the sulfate formation and the consequent increase of acidity.

VEGETATION EXPERIMENTS WITH SOIL 2

Similar experiments were made with soil 2, which was poorer in nitrogen and mineral food constituents. Soy beans were planted in the same manner as has been previously described, and sulfur, rock phosphate and acid phosphate employed at the same rates.

The changes in acidity were in this case quite similar to the changes in series I. After 3 weeks the plants were, in general, much poorer than the plants grown in series I. The leaves of nearly all plants had yellow spots, and some of them were more or less drooping. The general appearance and average height were considerably inferior after 6 weeks when 3 of the 6 plants from every culture were harvested, than was the case with plants grown in soil 1. The effect of sulfur was more pronounced and cultures receiving 1000 pounds of sulfur per acre were severely injured.

The soil responded far better to the treatment of phosphorus than the soil which had received fertilizers for the last 30 years. The cultures receiving rock phosphate produced, in general, higher yields than the cultures to which sulfur alone was added. In all cases again sulfur applications of 100 pounds to the acre gave slightly increased yields, while the others produced less dry weight.

All plants were scored for general appearance, yellowness and stockiness before harvesting. The scores show even more clearly than the dry weights of the plants that all cultures receiving 100 pounds of sulfur were better looking, greener and less spindling than the check plants or the plants grown in pots which received higher applications of sulfur.

A comparison of the nodules and root system brought out more definitely the same facts as were found in soil 1. The extent of the root system and also the number of nodules decreased with the increase of the sulfur applications, except on plants in cultures 2, 7, and 11, which were relatively greater.

Here again, a second crop of soy beans was planted in the same pots and all applications of sulfur, rock phosphate and acid phosphate were repeated.

The hydrogen-ion concentration in this second-crop experiment was considerably higher than in the case of the second crop on soil 1. The highest concentration was again found in culture 10, which received 300 pounds of rock phosphate to the acre besides 1000 pounds of sulfur. A number of the cultures were apparently less acid after 6 weeks than at the end of 5 weeks.

Notes taken a few days before harvesting show that the cotyledons of the cultures receiving 300 and 500 pounds of sulfur had in nearly all cases been dried out or dropped, while cultures receiving higher applications of sulfur had still dark green cotyledons. Culture numbers 3, 8 and 13 seemed most mature, while culture numbers 5, 10 and 15 were severely injured.

The dry weights and other data secured from the second crop are given in table 3.

TABLE 3

Yields of tops of second crop of soy beans grown in soil receiving no fertilizers for more than 30 years

POT NUMBER	DRY WEIGHT OF TOPS	RELATIVE WEIGHT	TOTAL TRANSPIRATION AND EVAPORATION	AVERAGE HEIGHT	NUMBER OF PODS	INITIAL REACTION	FINAL REACTION	REMARKS
				Treated with sulfur				
	gm.		*cc.*	*cm.*		*pH*	*pH*	
1	2.5990	100.0	3800	21.5	5	6.7	6.5	
2	2.4328	93.6	3500	21.6	5	6.7	6.2	
3	2.2447	86.5	3245	22.0	7	6.5	5.0	
4	1.9295	74.3	3110	19.0	5	6.0	4.9	
5	1.1808	45.4	2595	16.5	1	5.3	4.0	Two plants blooming, strongly injured
				Sulfur plus 300 pounds of rock phosphate				
6	2.3855	100.0	3665	22.0	9	6.9	6.3	
7	2.3700	99.5	3345	23.4	8	6.4	6.0	
8	2.1425	90.1	3435	21.5	4	6.3	6.0	
9	1.9261	80.8	2880	19.0	7	6.3	5.5	
10	1.1520	48.4	2715	13.5	2	6.1	3.8	Strongly injured
				Sulfur plus 100 pounds of acid phosphate				
11	2.2160	100.0	3395	21.5	10	6.7	6.4	
12	2.5500	114.6	3445	24.2	10	6.6	6.1	
13	2.1645	97.4	3330	22.4	8	6.6	6.0	
14	1.9410	87.3	3545	19.0	7	6.3	5.7	
15	1.9863	89.2	3320	18.5	5	6.3	4.5	Medium injured

It is apparent that plants grown in soil receiving acid phosphate in addition to sulfur were best. The whole series which received rock phosphate in addition to sulfur were poorer than the cultures receiving sulfur alone. The acidity produced in these pots did not seem to be enough to make the rock phosphate available, although some of the injury to the plants can be attributed to the acidity. Still this does not explain the phenomena in full. It is very likely, therefore, that the relatively high amounts of sulfates formed were harmful or prevented the plants from taking up the necessary food constituents. The results with this soil poor in nitrogen and mineral food constituents brings out clearly the fact that the acidity produced was not able to supply greater quantities of the necessary plant food elements.

The nodule formation was also influenced by the addition of sulfur, as can be seen by a relative comparison. If the check plants are placed at 100, the following figures were secured:

CULTURE NUMBER	NODULES	CULTURE NUMBER	NODULES	CULTURE NUMBER	NODULES
1	100	6	100	11	100
2	100	7	150	12	100
3	60	8	110	13	90
4	30	9	80	14	70
5	5	10	0	15	60

Sulfur added in large quantities depressed the formation of nodules considerably. The phosphate together with sulfur seemed either to have no influence, or were rather depressing. This may be seen if the relative numbers of nodules produced on the roots of plants to which sulfur alone was given are compared with the nodule formation on roots of plants which received phosphate in addition.

CULTURE NUMBER	NODULES	CULTURE NUMBER	NODULES	CULTURE NUMBER	NODULES
2	100	7	80	12	90
3	100	8	70	13	90
4	100	9	100	14	60
5	100	10	0	15	100

This seems to point toward a certain degree of stimulation by small additions of sulfur, but depression when the acidity produced becomes too great.

EXPERIMENTS IN SAND CULTURES

The acidity produced by inoculated sulfur in the soil cultures seemed insufficient to attack the rock phosphate added.

To test out whether or not a similar phenomenon of sulfate production from inoculated sulfur in the presence of rock phosphate could be intensified whereby the phosphorus would become more rapidly available, and to determine the effect of this intensified process on plant growth, soy beans were grown in washed quartz sand, using Shive's nutrient solution (R^5C^2) as a basis.

Florida soft rock phosphate was substituted for the phosphorus of the nutrient solution. The rock phosphate and inoculated sulfur had been mixed about 6 weeks previous to the application, kept in tumblers in an incubator, and tested for accumulation of acidity and for formation of sulfates. From these tests it was apparent that sulfur oxidation had begun. The mixture was added at the rate of 2 tons per acre which supplied approximately the calculated amount of phosphorus in the check (Shive's R^5C^2 solution) cultures.

Hydrogen-ion concentration determinations were made at the beginning of the experiment and regularly at the end of each week during the period in which the plants were growing.

The results obtained are given in table 4.

The inoculated sulfur with the rock phosphate mixed previous to the application which was substituted for the phosphorus in the cultural solution, had, at the end of 6 weeks, produced a fair growth of these plants. In the cases where inoculated sulfur was added the high acidity had killed the plants after about 14 days. The soy bean plants grown in Shive's nutrient solution to which inoculated sulfur was addded made a good growth at first, but in 3 weeks the plants, which untill then had survived, died. The roots were dark brown, with the appearance of being burnt.

Sulfur oxidation went on rapidly in the cultures to which the inoculated sulfur rock phosphate mixtures were added, as is indicated by the lowering of the pH values. Adding inoculated sulfur only to Shive's nutrient solution had also a decided influence on the lowering of the pH value, but no such great differences occurred as in the cultures to which previously mixed inoculated sulfur was added. This could be expected since sulfur oxidation had already begun in the mixture. Nevertheless, the plants grown in all cultures were killed in a comparatively short time. In the cases were no sulfur was added the plants made the soil solution less acid, bringing it nearly to the neutral point. This corresponds with the experiences in culture solutions where a similar phenomenon takes place. The changes in hydrogen-ion concentration were regular as is shown by the pH determinations, which were made at definite intervals.

TABLE 4

Results of growing soy bean plants in sand cultures for 6 weeks with inoculated sulfur and rock phosphate, and Shive's R_6C_2 solution as a basis

CULTURE NUMBER	TREATMENT	WEIGHT OF TOPS	AVERAGE LENGTH	NUMBER OF PODS	INITIAL REACTION	FINAL REACTION
		gm.	*cm.*		*pH*	*pH*
1	None	0.850	8	Bloom	5.8	6.8
3	Rock phosphate	1.454	11.5	Bloom	5 4	6.6
5	R_6C_2	3.610	15.0	4	5.3	6.6
6	R_6C_2, rock phosphate	2.223	15.0	2	5.0	6.4
8	R_6C_2, inoc. sulfur, rock phosphate	0.600	Dead		4.7	2.9
14	R_6C_2, inoc. sulfur	0.811	Dead		5.2	4 3

Apparently not sufficient acidity was produced in the soil cultures, since the acidity at which the phosphorus of the rock phosphate becomes soluble had not yet been reached. In the sand cultures this point was reached, but the plants were not able to survive. The fact that very little or no phosphorus is changed into a soluble form, has been found in the studies reported in another paper of this series. To find the exact acidity necessary to change the Florida soft rock phosphate used in these experiments into soluble P^2O^5, a curve was constructed from readings of pH values obtained by adding different amounts of 0.1 normal and normal sulfuric acid to the rock phosphate.

Ten grams of rock phosphate were shaken with definite amounts of sulfuric acid for 2 hours in a shaking machine and left standing overnight. An aliquot of the supernatant liquid was then drawn off and the hydrogen-ion determinations made. At the critical point sufficient determinations were made to check up all points obtained. The results are graphically shown in figure 1.

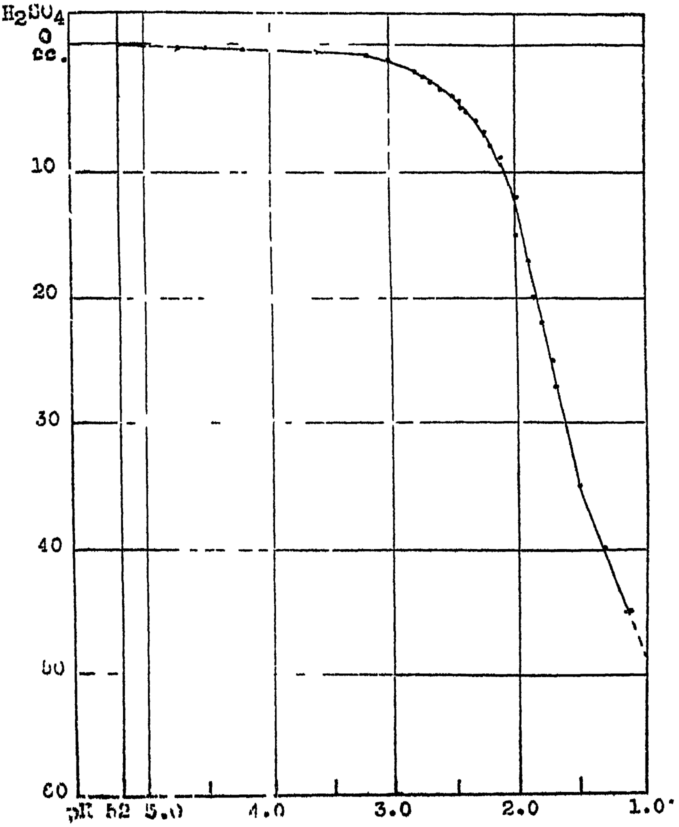


FIG. 1. GRAPH SHOWING THE HYDROGEN-ION CONCENTRATION AT WHICH ROCK PHOSPHATE BECOMES AVAILABLE

The curve shows that the phosphorus of the rock phosphate becomes rapidly available when a point is reached between pH 3.1 to 2.8.

Since under ordinary circumstances not sufficient acid is produced in the soil it is very doubtful whether greater beneficial effects can be expected from a mixture of rock phosphate and sulfur, which have not been composted, previous to the application, long enough so that most of the sulfur is transformed into sulfates and which has reacted with the phosphorus of the rock phosphate. It is very likely that the free acid produced by the sulfur oxidation is harmful to plant growth, more than the total amounts of sulfates or the so-called total acidity. Beneficial effects might be derived from inoculated sulfur which has not been composted with rock phosphate but mixed with the rock phosphate before applications are made, and when applied in small quantities, if the soil is in need of sulfur so that plant growth is stimulated. The plants under observation seemed to be stimulated by the sulfur to make more extensive root systems and thus would be able to take up greater amounts of plant food from the soil solution or from the slowly available rock phosphate present. At the same time the acidity produced would be not too intensive to be harmful to the normal development of roots and tops. It seems, however, more safe to compost the sulfur rock phosphate some time before application until most of the sulfur is converted into sulfates and has reacted with the phosphorus of the rock phosphate, in order to avoid the injurious effects.

BIOLOGICAL EXPERIMENTS WITH SOIL 1

In several instances it has been reported that sulfur and sulfates exerted a stimulating influence on bacterial growth. The possibly inadequate supply of sulfur in soils is apt to influence the microörganisms, and following the addition of certain quantities of it, a change in the biological flora can be expected.

The work reported in this part of the paper was mainly carried out to study the change in bacterial numbers and but slight attention was paid to the occurrence and determination of different species.

The amounts of sulfur added are given in table 5. During the incubation period hydrogen-ion concentration determinations of the soil water extract were made at frequent intervals. It was found that in all cases the hydrogen-ion concentration became greater until a certain point was reached, from then on the movement was back toward the neutral point. This turning point was reached in all cases before or between the ninth and the eleventh week of incubation. It should be remembered that no plants were growing in these tumblers. It seems, therefore, that the sulfates formed reacted with the soil constituents, bringing the soil solution back to a certain equilibrium. This phenomenon is even more clearly demonstrated by the curves shown in figure 2.

Bacterial numbers were determined by the plate method, using Lipman and Brown's synthetic agar, at the beginning of the experiment and after 6, 12 and 18 weeks. The numbers in thousands per gram of soil are given in table 5.

The bacterial numbers in the untreated soil remained about constant during the incubation period. The biological flora in the soils treated with amounts of sulfur up to 1500 pounds per acre seemed to be stimulated for the first 6 weeks, but the numbers in soils treated with greater quantities showed a rapid decline. The higher the applications of sulfur and consequently the higher the

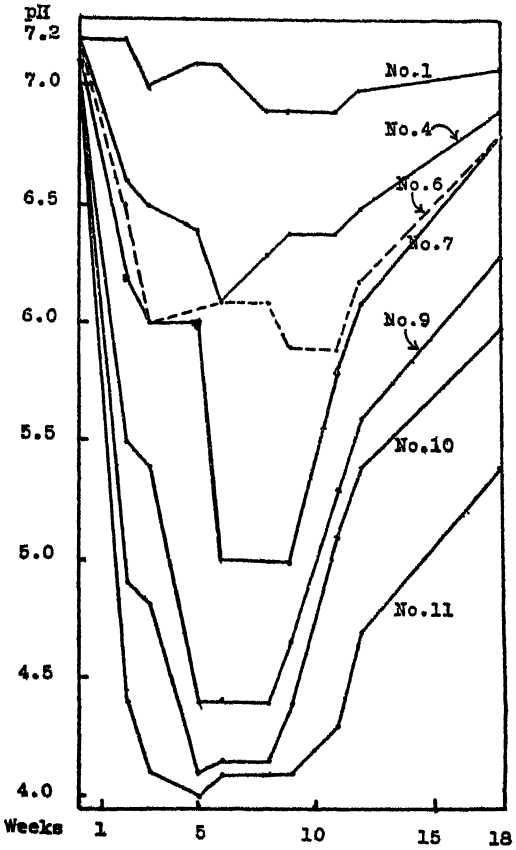


FIG. 2. CURVES SHOWING CHANGES OF HYDROGEN-ION CONCENTRATIONS OF SOIL 1 TREATED WITH DIFFERENT AMOUNTS OF INOCULATED SULFUR

TABLE 5

Bacterial numbers in soil 1 treated with inoculated sulfur

CULTURE NUMBER	SULFUR ADDED PER ACRE	NUMBER OF BACTERIA PER GRAM OF SOIL			
		Initial	After 6 weeks	After 12 weeks	After 18 weeks
	lbs.	*thousands*	*thousands*	*thousands*	*thousands*
1	None	3.280	3.195	3.200	3.150
2	100	3.240	2.520	3.525	3.475
3	300	3 190	3.470	3.615	3.535
4	500	3.200	4.470	3.325	3.225
5	1000	3.195	4.160	3.165	3.100
6	1500	3.260	3.600	2.720	2.950
7	2000	3.240	2.675	1.840	1.950
8	2250	3.275	2.390	1.415	0.850
9	2500	3.260	1.935	1.345	0.550
10	3000	3.235	1.405	0 765	0.450
11	3500	3.250	1.390	0.560	0.350

amounts of acidity produced, the more sulfates there were formed, the fewer colonies were counted on the agar plates. After 12 weeks much of the stimulating effect seemed to be lost in the cultures which had previously produced increased numbers. Applications of 100 and 300 pounds of sulfur to the acre seemed somewhat consistent in the production of higher numbers, but the decrease was very marked in the cultures receiving from 2000 pounds upward. The numbers in the cultures to which 3500 pounds of sulfur per acre was added had at the end of 12 weeks but slightly more than one-sixth of the original numbers. There is no doubt, however, that the depression of the original biological flora was accompanied by an extraordinary increase in sulfur-oxidizing organisms, but since these organisms do not reproduce on ordinary agar plated, or at least cannot be counted in the ordinary way on account of their extremely small size, no estimation of their numbers could be made.

After 18 weeks of incubation the amounts of sulfur oxidized were greater for all cultures which received the higher applications, but the movement of the hydrogen-ion concentration was still farther toward the neutral point. The numbers of colonies counted on agar plates increased in two cultures, while others showed a greater decrease. Cultures to which 3500 pounds of sulfur were added had at the end of 18 weeks but slightly more than one-tenth of the original numbers present.

After 24 weeks the majority of the cultures had reached the neutral point.

SUMMARY

Small amounts of inoculated sulfur had little or no influence upon the change of the hydrogen-ion concentration of soil 1, which had received fertilizers for about 30 years, but additions of larger quantities lowered the pH values.

The influence of sulfur oxidation was greater in soil 2, which had received no fertilizers for 33 years, and which was poorer in nitrogen and mineral plant food constituents than was soil 1.

Hydrogen-ion concentration increased nearly proportionally to the sulfur application.

Soils receiving rock phosphate in addition to the sulfur had usually higher hydrogen-ion concentrations than the soils in the pots receiving sulfur alone.

Soy bean plants, grown in soil treated with inoculated sulfur, were stimulated by small additions of sulfur, but were injured by larger additions.

Sulfur with acid phosphate in addition produced best soy bean plants, while the series receiving rock phosphate in addition were poorest.

The root systems of these soy bean plants were stimulated by small quantities of sulfur, but depressed by larger amounts.

Nodule formation seemed to be stimulated with small amounts of sulfur, but decreased numerically with the increase of the quantities of sulfur applied.

The phosphorus of Florida soft rock phosphate used in the experiments becomes available when a point in hydrogen-ion concentration between pH values 3.1 to 2.8 is reached.

The acidity produced by the oxidation of sulfur in these soils was not sufficient to render appreciable amounts of phosphorus more available, although the acidity produced was harmful to the soy-bean plants.

It is shown in sand cultures, that if sufficient acidity is produced to make the phosphorus available, the plants are killed.

Doubt is expressed whether greater beneficial effects can be expected from phosphate and inoculated sulfur mixtures, which have not been composted long enough previous to the application so that the sulfates and free acid formed have reacted with the phosphorus of the rock phosphate, than rock phosphate alone, unless the soil is in need of sulfur.

The hydrogen-ion concentration became greater in the uncropped soil with sulfur additions, until a certain point was reached; from then on the movement was back toward the neutral point.

The biological flora, expressed in numbers counted on agar plates from soil infusions, was slightly stimulated by small sulfur applications, but considerably depressed with larger amounts.

The formation of sulfates was influenced by the water content of the soil.

REFERENCES

(1) Ames, J. W., and Boltz, G. E. 1916 Sulfur in relation to soils and crops. Ohio Agr. Exp. Sta. Bul. 292.

(2) Bernhard, - 1910 Experiments on the control of potato scab. *Abs. in* Exp. Sta. Rec., v. 23, p. 744.

(3) Boulanger, E., and Dugardin, M. 1912 Mécanisme de l'action fertilisante du soufre. *In* Compt. Rend. Acad. Sci. (Paris), v. 155, p. 327–329.

(4) Brioux, Ch., and Geurbet, M. 1913 L'action fertilisante du soufre. Son évolution dans le sol. *In* Ann. Sci. Agron. 2, 1913, v. 2, p. 385.

(5) Brown, P. E., and Kellogg, E. H. 1914 Sulfofication in soils. Iowa Agr. Exp. Sta. Res. Bul. 18.

(6) Demolon, A. 1913 Récherches sur l'action fertilisante du soufre. *In* Compt. Rend. Acad. Sci. (Paris), v. 156, p. 725–728.

(7) Duley, F. L. 1916 The relation of sulfur to soil productivity. *In* Jour. Amer. Soc. Agron., v. 8, p. 154–160.

(8) Hart, E. B., and Peterson, W. E. 1911 Sulfur requirements of farm crops in relation to the soil and air supply. *In* Wis. Agr. Exp. Sta. Bul. 14.

(9) Headley, F. B. 1920 The work in 1918 of the Newlands reclamation project experimental farm. U. S. Dept. Agr. Cir. 80.

(10) Janicaud, W. 1914 Wirkt Schwefeldüngung wachstumsfördernd? *In* Gartenwelt, v. 18, p. 29–32.

(11) Lipman, J. G., McLean, H. C., and Lint, H. C. 1916 Sulfur oxidation in soils and its effects on the availability of mineral phosphate. *In* Soil Sci., v. 2, p. 499–538.

(12) Lyon, T. L., and Bizzell, J. A. 1918 Lysimeter experiments. N. Y. Exp. Sta. (Cornell) Mem. 12.

(13) Martin, W. H. 1921 A comparison of inoculated and uninoculated sulfur for the control of potato scab. *In* Soil Sci., v. 11, p. 75–85.

(14) Miller, H. G. 1919 Relation of sulfates to plant growth and composition. *In* J. Agr. Res., v. 17, p. 87–102.

(15) O'Gara, P. J. 1918 Available fertilizer from smelter by-products. *Abs. in* Exp. Sta. Rec. v. 41, p. 427.

(16) PITZ, W. 1916 Effect of elemental sulfur and of calcium sulfate on certain of the higher and lower forms of plant life. *In* Jour. Agr. Res., v. 5, p. 771–780.

(17) REIMER, F. C. 1914 Sulfur fertilizer for alfalfa. *In* Pacific Rural Press, v. 87, p. 717.

(18) REIMER, F. C., AND TARTAR, H. V. 1919 Sulfur as a fertilizer for alfalfa in Southern Oregon. Ore. Agr. Exp. Sta. Bul. 163.

(19) SHIVE, J. W. 1915 A study of physiological balance in nutrient media. *In* Physiol. Res., v. 1, p. 327–397.

(20) STEWART, R. 1920 Sulfur in relation to soil fertility. Ill. Agr. Exp. Sta. Bul. 227.

(21) SWANSON, C. O., AND MILLER, R. W. 1917 The sulfur content of some typical Kansas soils and the loss of sulfur due to cultivation. *In* Soil Sci., v. 3, p. 139–148.

(22) TACHEUCHI, T. 1916 The effect of sulfur and sulfur compounds on soil bacteria. *In* Ann. Bull. Imp. Inst. Tokyo., v. 14, p. 289.

(23) TOTTINGHAM, W. E., AND HART, E. B. 1921 Sulfur and sulfur compounds in relation to plant nutrition. *In* Soil Sci., v. 11, p. 49–73.

(24) VAN ALSTINE, E. 1920 The determination of hydrogen-ion concentration by the colorimetric method and an apparatus for rapid and accurate work. *In* Soil Sci., v. 10, p. 467–479.

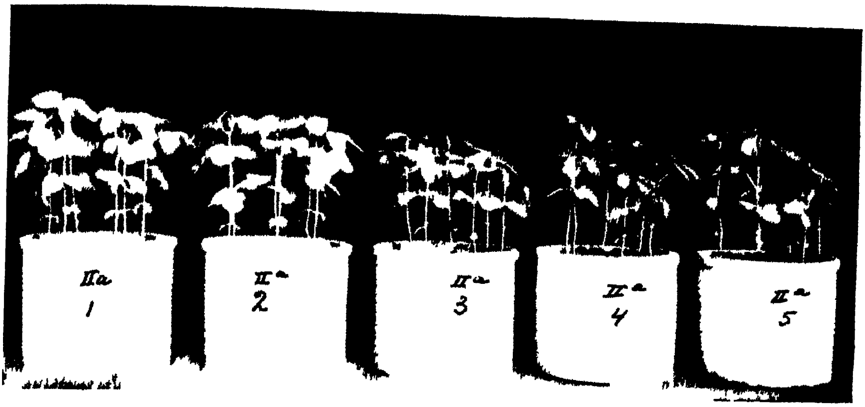


Fig. 1. Soy Bean Plants Grown in Soil Which Had Not Received Fertilizers for 30 Years. Sulfur Alone Added. No. 1 is Check

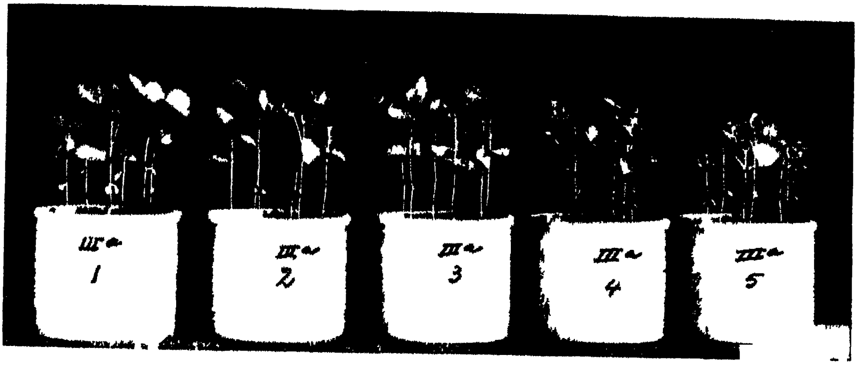


Fig. 2. Soy Beans Plants Grown in Soil Which Had Been Fertilized for More Than 30 Years to Which Sulfur Alone was Added. No. 1 is Check

CALCINED PHOSPHATIC LIMESTONE AS A FERTILIZER[1]

E. VAN ALSTINE

New Jersey Agricultural Experiment Stations

Received for publication, Nov. 1, 1921

INTRODUCTION

Before presenting experimental data it may be well to indicate the extent to which phosphatic limestone occurs, the quantities of phosphate held in it, and the significance which would attach to a method for profitable utilization of this source of phosphatic fertilizer.

RELATION BETWEEN PHOSPHATIC LIMESTONE AND PHOSPHATE ROCK

The brown phosphatic limestone from central Tennessee consists essentially of nodules of apatite varying in size from very fine dust to particles that will not pass a 20-mesh sieve, held together by a matrix of limestone, principally calcium carbonate. Where conditions have been favorable, the carbonates have been removed through solution in carbonated water and the much less soluble tricalcium phosphate is left but little affected except for the inevitable rounding off of sharp corners during the weathering process and except, also, for the deposition upon their surfaces of iron and aluminum oxides. This iron and aluminum existed in small amounts in the rock from which the phosphate has separated and the result of the weathering has been, through the removal of lime carbonate, a concentration, not only of the lime phosphate, but also of the iron and aluminum. Other substances, such as silica, which existed in the limestone and which are but slightly soluble in ground water remain mixed with the phosphate rock and reduce the tricalcium phosphate content to about 80 per cent or less. It sometimes happens, after the "brown rock" has been liberated from the limestone and concentrated, that it is more or less loosely bound together again by some cementing material which causes it to cohere in chunks of varying degrees of hardness when mined. These chunks will hold together well enough so that they can be built into piles over wood which is burned for the purpose of driving off moisture. Very much of the brown phosphate rock, however, exists as a loose granular deposit which forms a mud with water and which is easily removed from the ground with a shovel.

---

[1] Paper No. 43 of the Journal Series, New Jersey Agricultural Experiment Stations, Department of Soil Chemistry and Bacteriology.

This paper will appear in RUTGERS COLLEGE STUDIES, vol. 1.

Just why and how this phosphatic material came to be deposited with the limestone has not been definitely learned though it is supposed to have originated from the remains of some form or forms of marine life, but, since all known deposits of "brown rock" in central Tennessee have come from phosphatic limestone, these deposits, however extensive they may be, can exist only where conditions have been favorable for the dissolving action of ground water without the removal of phosphate material by erosion. Naturally, then, these deposits are limited in extent in comparison with the original limestone formation and, as a matter of fact, they are very limited in comparison with the still existing deposit of phosphatic limestone.

In many places the over burden of soil and rock has been so thick or so impervious, or has in other ways so protected the phosphatic limestone that it has not permitted this leaching. This condition still exists over extensive areas of the central basin of Tennessee and a study of any geological map of the area will show how extensive is this geological formation. Under this condition the phosphatic limestone may be covered by a thick layer of soil and rock or it may lie near the surface.

There are still other large areas where, during the most recent geological years, extending to the present time, erosion has been more than keeping pace with the removal of limestone by leaching. Where this erosion has not been so extensive as to remove the limestone itself in addition to what phosphate rock may have been liberated, the phosphatic limestone is left at or near the surface. A geological map shows that areas of this nature are also much more extensive than are the areas of workable deposits. It may reasonably be that the overlying strata have protected much of this limestone from leaching so that very little phosphate has been liberated. In this case the limestone has remained nearly or quite intact while it has continued to be uncovered by erosion.

In addition to the areas which exist under these two general conditions, there is also a considerable amount of phosphatic limestone which exists in or together with the workable deposits of phosphate rock. Rarely ever has the solution of limestone been complete. Even in those places where there are the best deposits of brown phosphate rock there exist unleached boulders and ledges of limestone termed "chimneys" or "horses" by the miners. Figure 1 shows the appearance of this limestone in a mine near Columbia, Tennessee after the phosphate rock had been removed.

The quantity and extent of the phosphatic limestone is many times greater than that of the phosphate rock. The quantity of phosphorus still locked up in the limestone in excess of that found in the rock phosphate deposits is roughly proportional to the excess of area still occupied by the phosphatic limestone over that occupied by the phosphate rock deposits.

Although new deposits of phosphate rock are still being found and opened up, the demand for the product has increased to such an extent that the methods of mining and purifying the phosphate have several times been improved to recover larger and larger percentages of the deposit.

PATENTS FOR THE UTILIZATION OF PHOSPHATIC LIMESTONE

Two patents (no. 971830 and no. 13302) aiming at the utilization of phosphatic limestone for fertilizer purposes have been taken out in this country. The plan of these patents is to make the phosphorus available to plants by burning phosphatic limestone at a temperature sufficiently high to drive off carbon dioxide and then slaking to break up the mass into a fine state of division. It has been claimed that this treatment, by breaking the phosphate nodules into a very fine state of division would make the phosphorus available.

More recently (2) a very similar German patent, no. 321776, has been granted. In this case a mixture of limestone and phosphate rock is burned.

Since limestone, heated to a temperature sufficient to drive off carbon dioxide slakes readily and reduces to a very fine powder, it remains only to determine how the phosphate in the phosphatic limestone is affected; to find out whether this too is pulverized, made porous or in any way made more available to plants.

During the summer of 1920 visits were made to a number of the worked phosphate deposits of central Tennessee and samples of phosphatic limestone were taken from several localities where there seemed to be an abundant supply of it easily accessible.

METHODS

In accordance with the method oulined in the American patents, quantities of these samples were broken into small pieces having a thickness of two inches or less and burned for ten hours at the full heat of a good gas furnace capable of holding six or eight pounds of the limestone when piled loosely with the muffle removed. The muffle was replaced by thin fire brick so that there was a reverbratory action of the flame. At the end of ten hours the lime was removed from the furnace and water was added to it while it was still hot and would slake readily. Only enough water was added to leave a comparatively dry powder when the slaking was complete.

This material, used in the tests reported below, was, for convenience, designated in the tables by the abbreviation HP and for brevity in discussions has been termed "hydrophos."

Under the treatment given, practically all of the carbon dioxide was driven off, as acid test showed, and the whole rock was reduced to what appeared from a superficial examination to be only hydrated lime. A sifting test showed, however, that a certain amount of it would not pass a 40-mesh sieve. One sample which had received the above treatment was rubbed well in a mortar with a rubber pestle. A sifting test of this showed about 12 per cent of the entire weight to be held on a 40-mesh sieve, 42 per cent on 100-mesh sieve, 18 per cent on a 200-mesh sieve and 28 per cent passing a 200-mesh sieve. Microscopical examination of the granules held on each of the sieves showed them to be coated with much finer particles of hydrated lime.

Another sample, after burning and slaking, was sifted and analyzed for the phosphorus content of the different separates. The results of the sieving and the analyses are shown in table 1 from which it may be seen that the composite sample after burning and slaking contained 23.5 per cent P_2O_5 equivalent to 51.35 per cent $Ca_3(PO_4)_2$.[2]

Microscopical examination in this case also showed hydrated lime adhering to all of the granules and yet the separates which did not pass a 100-mesh sieve contained more than one-half of the total phosphorus. The finer granules present a larger surface per unit weight to which hydrated lime may adhere.

TABLE 1

Sieving tests and phosphatic content of the separates from a sample of burned and hydrated phosphatic limestone

SIZE OF PARTICLES IN SEPARATES		WEIGHT OF SEPARATE		P_2O_5 IN SEPARATE	P_2O_5 AS FRACTION OF TOTAL WEIGHT	FRACTION OF TOTAL P_2O_5
Held by sieve	Passing sieve					
		gm.	*per cent of total*	*per cent*	*per cent*	*per cent*
40-mesh	20-mesh	25.9260	24.60	29.90	7.355	31.28
100-mesh	40-mesh	23.6708	22.46	29.20	6.558	27.89
200-mesh	100-mesh	13.3710	12.69	28.40	3.604	15.33
	200-mesh	42.4100	40.25	14.90	5.997	25.50
Totals................		105.3778	100.00		23.514	100.00

TABLE 2

Analysis of granular separate from burned and hydrated phosphatic lime-stone held on a 200-mesh sieve and thoroughly washed to remove free lime

CONSTITUENT	CONTENT
	per cent
Moisture at 100°C..	0.03
Total P_2O_5 [equivalent to 82.08 per cent $Ca_3(PO_4)_2$].................	37.60
Total CaO..	52.00
Total K_2O..	Trace
Iron oxides..	0.40
Aluminum oxides..	0.79
Silicon..	Trace

This is probably sufficient to explain the fact that the finer separates contain a higher percentage of lime and a lower percentage of phosphoric acid. The separate passing the 200-mesh sieve, of course, contained a very large amount of hydrated lime not adhering to phosphatic granules. Evidently there has been little, if any, pulverizing of phosphate granules by the process of treatment, for the phosphoric acid passing a 200-mesh sieve was but one-fourth of the entire quantity, an amount which may well have existed as granules of this degree of fineness in the untreated limestone.

---

[2] Chemical analyses, unless otherwise stated, were made by Wiley & Co., commercial chemists located in Baltimore, Md.

A sample of the burned and hydrated product passing a 40-mesh sieve and held by a 200-mesh sieve, after thorough washing and repeated rubbing with a soft rubber pestle to remove as much of the fine lime as possible, when dried and pulverized to pass a 100-mesh sieve, showed an analysis of 37.6 per cent of P_2O_5 which is equivalent to 82.08 per cent of tricalcium phosphate. This product has been designated by the abbreviation TP ("thermophos") and was used in series A of the following tests. A partial analysis of it showed the results presented in table 2.

Microscopical and chemical examination of samples of burned and hydrated phosphatic limestone revealed no difference due to differences in the length of time of burning, so long as the temperature was high enough and maintained long enough to drive off all of the carbon dioxide.

CULTURE EXPERIMENTS

Chemical and microscopical tests all giving negative results on the value of the material, it remained for culture experiments to show what results might be expected from its field use.

Sand Cultures, Series A

It was thought that results from sand cultures might be influenced less by factors not under control. Accordingly 138 cultures were prepared in 1-gallon stone jars holding 5 kgm. quartz sand which had been washed with running water to remove fine material, rinsed with distilled water and finally air dried. This sand had a water holding capacity of 24.48 per cent of the weight of the dry sand and during the growing period of the cultures the moisture content of the sand was kept close to 60 per cent of its water holding capacity (733 cc. per jar) by frequently weighing and adding the necessary amount of distilled water. Six of the cultures were treated with sulfur without beneficial effect and are not reported here.

Soy beans of the Edna variety were transplanted to these jars, six plants to a culture on October 30, 1920. The number of plants per jar was reduced to three on November 30 and the final harvest was made on December 29. Weights were taken on the plants harvested November 30, but the results showed no significance which was not more strikingly shown in the results of the second harvest and, therefore, will not be reported.

On December 8, about six weeks after planting, solution was drawn from the bottom of each jar and its hydrogen-ion concentration determined by the colorimetric method. The pH values obtained varied from 5.0 to 8.5, but there was no relation between these values and yields or availability of the phosphate fertilizers.

With the following explanations the treatment of each of the cultures, as indicated in table 4 and the accompanying foot notes, will be easily understood. Except for the last 8 cultures (131–138), all the essential plant food elements were added in the form of salts in solution. The salts used and the quantities of half molecular solutions per liter of the 733 cc. of moisture added to each jar at the beginning of the experiment were as shown in table 3.

The quantities of salts used and the volume of water added at the beginning were such as to make a solution having an osmotic pressuse value of about two atmospheres.

As a basis for the quantities of phosphorus to be applied, acid phosphate analyzing 17.8 per cent total P_2O_5 was applied in four different quantities equivalent to 200, 500, 1000 and 2000 pounds per 2,000,000 pounds of sand. Where phosphate fertilizers of other forms were used they were added in quantities which would supply the same amounts of phosphorus.

Burned and hydrated phosphatic limestone pulverized to pass a 100-mesh sieve contained 23.85 per cent of total P_2O_5 and 33.5 per cent of $Ca(OH)_2$ while the product obtained by sifting the unpulverized hydrated product through a 200-mesh sieve contained 13.76 per cent P_2O_5 and 51.29 per cent of $Ca(OH)_2$. For the purpose of comparison certain cultures without phosphorus and certain others with each of the forms of phosphate fertilizers other than the above were treated with hydrated lime in amounts equal to those contained in corresponding treatments with the above phosphate products. These facts are indicated in table 4 by the abbreviations $HL^1 - HL^4$ and $HL^5 - HL^8$.

TABLE 3

Nutrient salts and the amounts used in the sand cultures with soy beans

NUTRIENT SALTS USED	0 5 M SOLUTION PER LITER OF SOLUTION ADDED AT START	WEIGHT OF SALTS PER JAR
	cc.	*gm.*
$MgSO_4$	40.60	1.79
KNO_3	34.84	1.29
$Ca(NO_3)_2$	5.08	0.31

Calcium cabonate in the form of a high grade limestone pulverized to pass a 100-mesh sieve and in amounts equal to those found in the four quantities of pulverized phosphatic limestone used, was added to some of the jars without posphorus and to some of the jars receiving each of the other forms of phosphatic fertilizers except burned phosphatic limestone ("HP").

As a check to see whether or not the burning of rock phosphate would cause it to have the same effect upon crop production that the burning might cause the phosphate in the limestone to have, a quantity of floats was burned for 10 hours at a temperature of about 800°–1000°C. and used in comparison with the other phosphates.

Since there seemed to be but little, if any, difference in yields when different quantities of the same phosphate materials have been used, yields for the treatments with the same materials have been averaged together as reported in table 4.

The probable error (1) of the relative yield values in tables 4 and 5 has been calculated in each case upon original weights of individual plants by the formula

$$p.e. = 0.6745 \frac{d^2}{n(n-1)}$$

The probable error of the percentage increases has been calculated in each case upon original weights by the formula for probable error of difference

$$p.e. = e^1 - e^2 \pm \sqrt{(a^1)^2 + (a^2)^2}$$

Symbols used in table 4

GL^1, GL^2, GL^3, GL^4 = ground limestone (88.41 per cent $CaCO_3$) at rates of 0.1708, 0.4270, 0.8540 and 1.7800 gm. per jar furnishing calcium carbonate equal to that added to the jars in PL^1, PL^2, PL^3, PL^4 respectively.

HL^1, HL^2, HL^3, HL^4 = hydrated lime (97.26 per cent $Ca(OH)_2$) at rates of 0.1402, 0.3506, 0.7011 and 1.4023 gm. per jar, supplying $Ca(OH)_2$ equal to that added in HP^1, HP^2, HP^3, HP^4.

HL^5, HL^6,HL^7, HL^8 = hydrated lime at rates of 0.5045, 1.2612, 2.5224 and 5.0449 grams per jar supplying $Ca(OH)_2$ slightly in excess of that added in HP^5, HP^6, HP^7, HP^8.

PL^1, PL^2, PL^3, PL^4 = phosphatic limestone (21.76 per cent total P_2O_5) pulverized to pass a 100-mesh sieve and used in quantities supplying phosphorus equal to that added in AP^1, AP^2, AP^3, AP^4.

HP^1, HP^2, HP^3, HP^4 = burned and hydrated phosphatic limestone (23.85 per cent total P_2O_5) pulverized to pass a 100-mesh sieve and used in quantities supplying phosphorus equal to that added in AP^1, AP^2, AP^3, AP^4.

HP^5, HP^6, HP^7, HP^8 = the fine separate passing a 200-mesh sieve from burned and hydrated phosphatic limestone (13.76 per cent total P_2O_5) used in quantities supplying phosphorus equal to that added in AP^1, AP^2, AP^3, AP^4.

TP^1, TP^2, TP^3, TP^4 = phosphatic limestone, burned, hydrated, washed on a 200-mesh sieve, elutriated until nearly free from $Ca(OH)_2$, pulverized to pass an 100-mesh sieve (37.6 per cent total P_2O_5) and used in quantities supplying phosphorus equal to that added in AP^1, AP^2, AP^3, AP^4.

RP^1, RP^2, RP^3, RP^4 = floats (34.1 per cent total P_2O_5) pulverized to pass a 100-mesh sieve and used in quantities supplying phosphorus equal to that added in AP^1, AP^2, AP^3, AP^4.

RP^5, RP^6, RP^7, RP^8 = floats passing a 200-mesh sieve (33.25 per cent total P_2O_5) used in quantities supplying phosphorus equal to that added in AP^1, AP^2, AP^3, AP^4.

BRP^1, BRP^2, BRP^3, BRP^4 = floats (34.94 per cent total P_2O_5) pulverized to pass a 100-mesh sieve, burned for ten hours at about 800° to 1000°C. and used in quantities supplying phosphorus equal to that added in AP^1, AP^2, AP^3, AP^4.

AP^1, AP^2, AP^3, AP^4 = acid phosphate (17.8 per cent total P_2O_5) used at the rates of 200, 500, 1000 and 2000 pounds per 2,000,000 pounds of dry sand.

BS^1, BS^2, BS^3, BS^4 = basic slag (18.25 per cent total P_2O_5) used in quantities supplying phosphorus equal to that added in AP^1, AP^2, AP^3, AP^4.

Results of Series A

From table 4 it may be observed that all the forms of phosphate produced some increase over growth obtained without phosphorus and without lime in some form, but none of the forms of phosphorus, except acid phosphate and basic slag, produced under any condition more growth than was obtained with ground limestone without phosphorus.

TABLE 4

Relative yields and probable error of soy beans in series A and percentage increase with probable error of difference for cultures treated with phosphorus over those without phosphorus

CULTURE NUMBERS	PHOSPHORUS TREATMENT	RELATIVE YIELD VALUES UNDER VARIOUS ADDITIONAL TREATMENTS†				
		No additional treatment	KN§	KN§ GL^1-GL^4	KN§ HL^1-HL^4	KN§ HL^5-HL^8
135–138	None	10 ±0.4				
1– 4	None		25 ±0.8			
5– 8	None			39 ±1.9		
9– 12	None				35 ±1.9	
13– 16	None					32 ±1.2
17– 20	PL^1-PL^4	(37 ±1.2)*	37 ±1.2			
Increase for PL^1-PL^4		*268%±13%**	*45%±6%*			
131–134	HP^1-HP^4	19 ±0.6				
22– 25	HP^1-HP^4		35 ±1.4		(35 ±1.4)*	
Increase for HP^1-HP^4		*95%±7%*	*41%±7%*		*3%±5%*	
26– 29	HP^5-HP^8		37 ±1.0			(37 ±1.0)*
Increase for HP^5-HP^8			*45%±5%*			*15%±5%*
31– 34	TP^1-TP^4		33 ±1.1			
36– 39	TP^1-TP^4			36 ±1.1		
41– 44	TP^1-TP^4				36 ±2.0	
45– 48	TP^1-TP^4					35 ±1.4
Increase for TP^1-TP^4			*30%±5%*	*−7%±6%*	*4%±7%*	*8%±6%*
49– 52	RP^1-RP^4		29 ±1.6			
54– 57	RP^1-RP^4			37 ±1.4		
59– 62	RP^1-RP^4				38 ±1.6	
63– 66	RP^1-RP^4					37 ±1.0
Increase for RP^1-RP^4			*16%±7%*	*−6%±6%*	*10%±6%*	*16%±5%*

67– 70	RP^5-RP^8		34 ±1.5			
71– 74	RP^5-RP^8			38 ±1.3		
75– 78	RP^5-RP^8				36 ±1.8	
79– 82	RP^5-RP^8					36 ±0.8
Increase for RP^5-RP^8			*35%±7%*	*−2%±6%*	*5%±6%*	*12%±5%*
83– 86	BRP^1-BRP^4		32 ±1.9			
87– 90	BRP^1-BRP^4			37 ±0.7		
91– 94	BRP^1-BRP^4				39 ±1.9	
95– 98	BRP^1-BRP^4					32 ±1.0
Increase for BPR^1-BRP^4			*27%±8%*	*−6%±5%*	*14%±6%*	*2%±5%*
99–102	AP^1-AP^4		78 ±3.6			
103–106	AP^1-AP^4			100 ±3.7‡		
107–110	AP^1-AP^4				95 ±3.4	
111–114	AP^1-AP^4					68 ±2.3
Increase for AP^1-AP^4			*209%±15%*	*156%±11%*	*175%±10%*	*115%±8%*
115–118	BS^1-BS^4		87 ±3.2			
119–122	BS^1-BS^4			76 ±1.8		
123–126	BS^1-BS^4				66 ±1.6	
127–130	BS^1-BS^4					53 ±2.6
Increase for BS^1-BS^4			*247%±13 %*	*95%±7%*	*91%±5%*	*66%±9%*

---

* Percentage increases with probable error of difference have been calculated on the actual weights taken rather than on the relative yields given in this table.

† All cultures were inoculated with a few cc. of a water-extract of soil from a soy bean field where nodule-forming bacteria were abundant, and were supplied with iron as the chloride and as the sulfide.

‡ Average weight of plant was 1.8968 gm.

§ KN is an abbreviation used to indicate a solution of salts supplying nitrogen, potassium, calcium, magnesium and sulfur as indicated in tabe 3.

Values in parentheses have been carried forward or back from "KN" column to the columns of other values with which they may properly be compared.

When used in addition to limestone or hydrated lime, neither "hydrophos" nor "thermophos" proved to be of more value than rock phosphate. The larger amounts of hydrated lime (HL^5 — HL^8) seem to have decreased plant growth, especially when used in addition to acid phosphate or basic slag. Cultures receiving acid phosphate except those to which the larger amounts of hydrated lime were added, produced two to four times as much growth as any other cultures except those treated with basic slag, while the corresponding cultures with basic slag produced one and two-thirds to two and one-third times as much growth as cultures treated with any other form of phosphorus except acid phosphate, comparing always those cultures which receive like treatment in addition to phosphorus. In no case with any of the phosphates used other than acid phosphate or basic slag, was the increase or decrease great enough or the probable error of difference small enough to indicate with any degree of certainty that the variation in yield from that of the corresponding culture without phosphorus was due to anything other than to individuality in plant growth.

The material prepared by simply burning and hydrating phosphatic limestone was not used with limestone sufficient to neutralize the soil acidity because it was thought that the phosphorus from this source would be more available in an acid soil. It is not probable that the difference in yield with and without limestone would have been greater than that for floats with and without limestone.

Sulfur inoculated with sulfur-oxidizing organisms, was added to six cultures with the hope that the acid produced would dissolve phosphorus and make it available. The amount added was 0.0522 gram per jar, equal to one-fifth the quantity of floats (RP^1) with which it was used in two of the cultures. None of the cultures so treated produced greater growth over the corresponding cultures without sulfur than may be accounted for by experimental error.

Soil Culture, Series B.

In addition to the sand cultures, another series of cultures was grown in soil. The soil was taken from the surface foot of a sassafras silt loam, from a field which had not been fertilized for 30 years or more and had not been under cultivation for a number of years. It contained 0.098 per cent of total P_2O_5 and had a lime requirement, according to the Veitch test, of 2000 pounds of CaO per 2,000,000 pounds of soil. Loss on ignition was 6.13 per cent and its hydrogen ion concentration at the beginning of the experiment corresponded to a pH value between 5.5 and 5.9. Its water holding capacity was found to be 46.86 per cent of the weight of the dry soil and during the growth of the cultures the soil was kept near 57 per cent of its water holding capacity. During the first part of the experiment the cultures were weighed when water was added, but towards the end they were watered without weighing.

Series B was soy beans of the Edna variety, four plants to a culture, grown from April 30 to July 2, 1921, in 2 gallon jars containing 7500 grams of air dry soil.

As in the sand culture series, acid phosphate was taken as the standard of phosphorus application, 400 pounds per 2,000,000 pounds of soil being the rate of application. Applications of other phosphate fertilizers were such that the amount of phosphorus added equaled that of the acid phosphate applications.

One culture was left without fertilizer treatment of any kind while the others received combinations of lime and a fertilizer mixture supplying nitrogen and potassium. Each of the cultures was inoculated with the proper legume bacteria. Details as to the kind and amounts of salts used and also other details of treatment are given in footnotes to table 5.

Symbols used in table 5

GL^1, GL^2, GL^4 = calcium carbonate (c.p.) used at rates of 1,000, 2,000 and 4,000 pounds of CaO per 2,000,000 pounds of soil respectively. The Veitch test showed 2,000 pounds of CaO to be necessary to neutralize the acidity of 2,000,000 pounds of the soil.

ML^1, ML^2 = magnesium carbonate (c.p.) used at rates equivalent to 1,000 and 2,000 pounds of CaO per 2,000,000 pounds of soil.

$(CaSO_4)^1$, $(CaSO_4)^2$ = calcium sulfate (c.p.) supplying Ca at rates equal to 1,000 and 2,000 pounds of CaO per 2,000,000 pounds of soil.

KN = potassium sulfate (c.p.) and sodium nitrate (c.p.) each used at the rate of 200 pounds per 2,000,000 pounds of soil.

K = potassium sulfate (c.p.) used at the rate of 200 pounds per 2,000,000 pounds of soil.

NO_3 = sodium nitrate (c.p.) used at the rate of 200 pounds per 2,000,000 pounds of soil.

NH_4 = ammonium sulfate (c.p.) used at the rate of 155 pounds per acre supplying nitrogen at a rate equivalent to 200 pounds of sodium nitrate per 2,000,000 pounds of soil.

HP = "hydrophos," phosphatic limestone burned ten hours, slaked with water while hot, pulverized to pass an 100-mesh sieve (23.85 per cent total P_2O_5) and used without further treatment at the rate of 298 pounds per acre supplying phosphorus at a rate equivalent to 400 pounds of acid phosphate per 2,000,000 pounds of soil.

AP = acid phosphate (17.8 per cent total P_2O_5) used at the rate of 400 pounds per 2,000,000 pounds of soil. Analysis showed the soil to contain .098 per cent total P205.

RP = floats (34.1 % total P_2O_5) pulverized to pass an 100-mesh sieve and used at the rate of 208 pounds (equivalent to 400 pounds of acid phosphate) per 2,000,000 pounds of soil.

OM = organic manure (fresh horse dung) used at the rate of ten tons per 2,000,000 pounds of soil.

KP = potassium acid phosphate ($KH_2(PO_4)_2$, c.p.) used at the rate of 3264 pounds per 2,000,000 pounds of soil.

CaP = calcium acid phosphate ($CaH_4(PO_4)_2$, c.p.) used at the rate of 2568 pounds per acre, supplying P_2O_5 at 91.45 % the rate of 3264 pounds of $KH_2PO_4)_2$ and supplying CaO at the rate of 615 pounds per acre, equivalent to 614 pounds of CaO per acre.

Results of Series B

The relative weights of dry tops for each treatment of series B are brought together in table 5 along with the probable error for each of them determined on the weights of individual plants. As indicated in table 5 these figures are in some cases averages of two cultures and in others they are of single cultures.

TABLE 5

Relative yields and probable error of soy beans in series B and percentages increase with probable error of difference for cultures treated with phosphorus over those without phosphorus*

CULTURE NUMBERS	pH VALUES OF SOIL AT HARVEST	ADDITIONAL TREATMENT†	PHOSPHORUS TREATMENT								
			None	HP		AP		RP		KP	CaP
				Yield	Gain*	Yield	Gein*	Yield	Gain*		
					per cent		*per cent*		*per cent*		
1, 11	5.7	None	17.8±2.3								
2, 12	6.8, 6.9	GL^4	16.1±0.7								
3, 4	6.8, 6.9	GL^4, NO_3, K	15.7±0 9								
5, 6	6.9	GL^4, NO_3, K		15.6±0 3	−1± 6						
7, 8	6.9, 6.8	GL^4, NO_3, K				20.7±0.6	32± 7				
9, 10	6.8, 6.9	GL^4, NO_3, K						16.3±0.5	4±6		
13, 14	6.7	GL^4, NH_4, K	16.5±0 8								
15, 16	6.8, 6.7	GL^4, NH_4, K		18.1±0.7	10± 7						
17, 18	6.7	GL^4, NH_4, K				24.5±1.5	49±11				
19, 20	6.7	GL^4, NH_4, K						16.7±1.3	1±9		
37	6.8	GL^2, ML^2, NO_3, K		38 4±0.4							
21	6.4	GL^2	29.7±2.3								
24	6.4	GL^2, NO_3, K	33.1±2.5								
25	6.6	GL^2, NO_3, K		35.5±3.5	7±13						
26	6.6	GL^2, NO_3, K				45 0±5.5	36±18				
27	6.4	GL^2, NO_3, K						32.5±2.6	−2±11		
22, 23	6.4	GL^2, OM	76.2±4.0								
33	6.3	ML^2	35.9±3.5								

34	6.0	ML^2, NO$_3$, K	42.0±6.8								
35	6.3	ML^2, NO$_3$, K		42.3±2.5	1±18						
36	6.0	ML^2, NO$_3$, K, (CaSO$_4$)^2		35.8±0.6							
42	6.5	GL^1, ML^1, NO$_3$, K		43.5±2.9							
28	6.1	GL^1	41.4±2.5								
29	5.9	GL^1, NO$_3$, K	42.1±3.6								
30	5.9	GL^1, NO$_3$, K		47.6±6.9	13±18						
31	6 0	GL^1, NO$_3$, K				52.0±3.4	23±12				
32	6.0	GL^1, NO$_3$, K						40.3±2.4	−4±11		
38	6 0	ML^1,	45.4±3.3								
39	6.0	ML^1, NO$_3$, K	48 0±3.3								
49	5 9	ML^1, NO$_3$, K		48.3±3.0	1±10						
41	5.9	ML^2, NO$_3$, K, (CaSO$_4$)^2		48.6±4.7							
45	6.2	GL^2, NO$_3$								100.0±2.7‡	
43	5.8	NO$_3$								68.5±1.2	
44	5.7	NO$_3$, (CaSO$_4$)^2								67.7±2.2	
46	5.6	NO$_3$, K									60.5±8.8

---

* Percentage increases with probable error of difference have been calculated on the actual weights rather than on the relative yields given in this table.

† All cultures were inoculated with the proper nodule-forming bacteria.

‡ Average weight of plants was 14.568 gm.

Percentage increase or decrease with probable error of difference has also been calculated on weights of individual plants. The pH values included in this table were determined on samples of soil taken from each jar at about harvest time.

The response which this soil shows to heavy applications of phosphorus is indicated by the weight of culture 45, with 0.1623 per cent of KH_2PO_4, which produced the heaviest crop of all, and by the weights of cultures 43, 44 and 46, also with large applications of phosphorus, which produced the next heaviest yields, excepting only cultures 22 and 23 which were heavily fertilized with organic manure.

As table 5 shows, there are six possible comparisons between cultures without phosphorus fertilizer and cultures receiving "hydrophos." There are four similar comparisons possible for the effect of rock phosphate. In these ten comparisons the relation between percentage increase and the corresponding probable error of difference is such that in only one case is there so much as an even chance that either "hydrophos" or rock phosphate used at rates equivalent to 400 pounds of acid phosphate per acre produced an increase in growth. Acid phosphate produced increases large enough to be beyond question of doubt.

Barley Cultures, Series C

A third series of cultures consisted of barley grown in soil from the same source as that used in series B. Acid phosphate was used in amounts equivalent to 200 and to 400 pounds per 2,000,000 pounds of soil and both "hydrophos" and floats were used in corresponding amounts. The results obtained agreed with those from series A and series B in showing a decided increase in growth from acid phosphate, but no decided effect from either "hydrophos" or rock phosphate. Figure 3 shows a few cultures from this series the treatment of which varied only in phosphate fertilizer.

CONCLUSIONS

1. Burned and hydrated phosphatic limestone is considerably inferior to acid phosphate as a fertilizer either in sand cultures or in soil cultures.
2. The amount of hydrated lime supplied by using burned phosphatic limestone in amounts corresponding to 400 pounds of acid phosphate to the acre is entirely ineffective in correcting the lime requirement of an acid soil.
3. Any advantage that burned and hydrated phosphatic limestone may show over phosphate rock is so slight that it may be accounted for by the fact that its iron and aluminum content is lower and is in less intimate contact with the tricalcium phosphate.
4. Pulverized phosphatic limestone is as valuable for soil treatment as are any of the phosphatic products obtained in this test from the burning of this limestone.

REFERENCES

(1) Merriman, Mansfield 1913 A Text Book on the Method of Least Squares. Wiley and Sons, New York.

(2) Stoppani, E., and Volpato, V. 1921 Disintegration of mineral phosphates for fertilizer manufacture. *In* Chem. Abs., v. 15, no. 10, p. 1595.

PLATE 1

Fig. 1. Phosphatic Limestone in a Phosphate Rock Mine Near Columbia, Tennessee, after the Overlying Soil and the Phosphate Rock had Been Removed

Fig 2. Sand Cultures from Series A

Culture 107 with acid phosphate.
Culture 25 with burned and hydrated phosphatic limestone ("HP").
Culture 44 with the washed granular phosphate from the burned and hydrated phosphatic limestone ("TP")
Culture 62 with 100-mesh floats
Culture 78 with 200-mesh floats
Culture 123 with basic slag

Fig. 3. Barley Cultures in Soil from Series C—Fertilizer Salts (KN) and also the Phosphates Added to the Soil for a Previous Crop of Soy Beans

Culture 38 without phosphorus
Culture 41 without phosphorus
Culture 6 with "hydrophos" (HP^2).
Culture 16 with acid phosphate (AP^2)
Culture 25 with floats (RP^2)
Culture 34 with c.p. tricalcium phosphate (PP^2).

FIG. 1

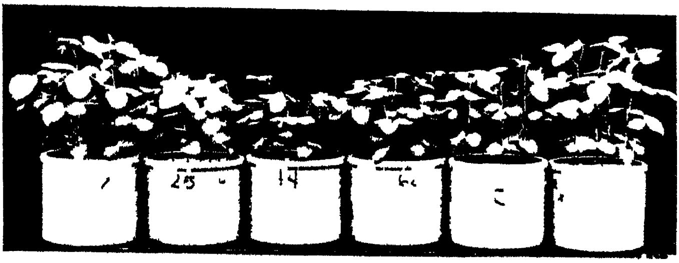


FIG. 2

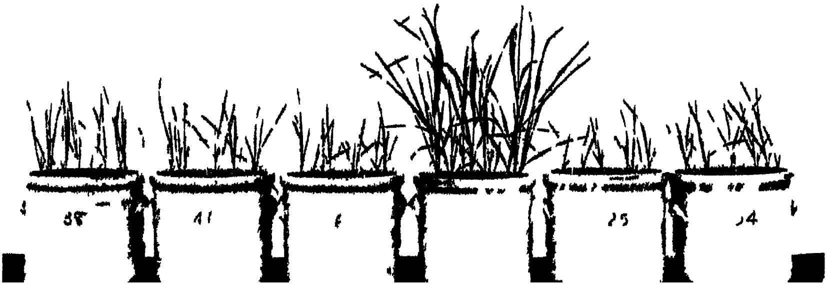


FIG. 3

MICROBIOLOGICAL ANALYSIS OF SOIL AS AN INDEX OF SOIL FERTILITY: II. METHODS OF THE STUDY OF NUMBERS OF MICROÖRGANISMS IN THE SOIL[1]

SELMAN A. WAKSMAN

New Jersey Agricultural Experiment Stations

Received for publication, December 6, 1921

INTRODUCTORY

In taking up the study of bacterial numbers in the soil as the first function for the characterization of the bacteriological condition of the soil, one should keep in mind that this is done not because it is the most important function, nor because it is known to be the most representative function, but for several other reasons. Following the determination of bacterial numbers in the soil by Koch in 1881 with the introduction of the gelatin plate, the first attempt was then made to make a thorough study of bacteria in the soil. With the possible exception of the decomposition of nitrogenous organic matter (ammonification) and nitrification, more work has been done in the study of numbers of bacteria in the soil as influenced by various factors, than of any other soil bacteriological function. This is a simple function, readily placed on a quantitative basis and does not have the complex qualitative character of the most other functions, which have not yet been placed fully on a quantitative basis. Our methods for determining bacterial numbers in the soil are well worked out, the limitations involved are well recognized and the variability factor can be readily calculated.

A historical review of the occurrence and distribution of bacterial numbers in the soil is found in the work of Voorhees and Lipman (17, p. 10–12), Löhnis (11) and various papers dealing with the subject Brown (2), Conn (4), Waksman (18), etc.

The determination of numbers of microörganisms in the soil has not been looked upon as of prime importance in the study of its bacteriological condition. The results obtained have been very variable, non-uniform and not very promising for the interpretation of soil fertility phenomena. A lack of confidence has been felt on the part of even the trained bacteriologist, who has recognized the limitations of the methods and found himself unable to correlate the results obtained by the determination of numbers of microörganisms in the soil with its crop producing power. This has led bacteriologists (Remy, 14) to state that the number of bacteria in any soil has but a very limited diagnostic value in ascertaining its fertility. This statement of Remy was based merely upon the fact that the number of colonies of microörganisms on the plate shows no direct relationship to the ammonifying, nitrifying or denitrifying power of the soil. It remains to be proved however, whether these last mentioned physiological activities are of diagnostic value. This untrustworthiness was also pointed out by Löhnis (10) who called attention to a difference in a small number of efficient bacteria and a large number of organisms possessing only a slight efficiency. He further points to the determination of bacteriological numbers in the soil as "rather worthless and spirit-destroying countings." In a later publication (12), Löhnis emphasizes this fact by stating that "all further investigations have shown, without any exception, that no definite relationship between the total number of bacteria and fertility of corresponding soil is recognized." Even as late as 1921, we find such a striking statement as "a quantitative bacteriological analysis of soil for total numbers of microörganisms has but a comparatively small significance as compared with that for the estimation of numbers in one or more physiological groups" (Northrup-Wyant, 13). Such a severe criticism of the value of determining the total numbers of microörganisms in the soil has been justified by the great variability in bacterial numbers reported by various soil bacteriologists and the lack of correlation between the numbers and the physiological activities of specific groups of microörganisms in the soil which are assumed to be of great importance to soil fertility (Remy, 14).

---

[1] Paper No. 81 of the Journal series, New Jersey Agricultural Experiment Stations, Departmen of Soil Chemistry and Bacteriology.

This paper will appear in RUTGERS COLLEGE STUDIES, vol. 1.

However, not all investigators have reached such negative conclusions as to the value of determining bacterial numbers in the soil. We may but refer to the work of Russell and Appleyard (16), who found the curves for bacterial numbers, nitrate content and carbon dioxide in the soil to be sufficiently similar to justify the view that all the phenomena are related.

The physiological activities of soil microörganisms will be taken up in the following papers, while here, we will limit ourselves only to numbers. A study of the variability and methods of mathematical interpretation of bacterial numbers has been reported in the previous paper (19) in this series. In this latter paper the author has endeavored to show that the variability and lack of correlation mentioned above are due to lack of uniformity in the methods, inaccurate methods, and changing soil flora. Nonuniformity of methods is made even worse by the fact that the details of the technic used by the various investigators are so widely divergent and that the data obtained even from one soil by the various methods are incomparable. Each investigator is, in the words of Northrup-Wyant (13), "a law unto himself in so far as the technic used in quantitative bacteriological soil analysis is concerned." Great variability in the numbers obtained on the same soil by the same investigator may also be due to the fact that the results are not checked sufficiently and the probable errors are too great for any accurate scientific work. This has been well recognized by various bacteriologists such as Chester (3).

METHODS

Although the methods used in a quantitative bacteriological analysis of soil have been reviewed in several of the more recent publications, as pointed out above, the various limitations of the methods have not been studied sufficiently, so that the ground is only incompletely covered. To determine just how much weight should be attached to the methods by which a particular soil phenomenon (function) is measured in comparison with the other soil microbiological activities (functions) in the building up of a system of soil bacteriology, a study of methods is of prime importance.

The various methods used for determining quantitatively the microörganisms in the soil can be classified into 3 groups:

1. Dilution method
2. Plate method
3. Direct counting method

The dilution method can be utilized not only for the determination of the total soil flora but also for the study of specific physiological groups, utilizing differential media. But the use of this method in routine bacteriological soil analysis is too cumbersome, involving a number of dilutions and cultures for each soil, and then only approximate results would be obtained, particularly in view of the fact that the selective action of the medium would be as manifest as in the case of the plate method, since not all organisms would develop on any one medium. The microscopic method suggested by Conn (5) gives promising results, but has not yet been developed to a sufficient extent to warrant any definite conclusions. The great difference between the plate and microscopic counts in normal soil is due to organisms which cannot be grown on plates. In the case of those organisms that develop on plates, the plate count will be nearly as high or even higher than the microscopic count, according to Conn. If we keep in mind, therefore, the limitations of the plate method, we will find it quite satisfactory for our work, until the direct counting method is developed sufficiently to warrant its exclusive use.

The plate method has been the one most commonly employed by bacteriologists, and has also been used in the present investigations. It is an indirect method, since we do not count the organisms directly, only the colonies that are produced on the plate. It is assumed that every bacterium, actinomyces, fungus spore or hypha develop into a colony. This can be justified only when the organisms are well separated from the soil into the diluting fluid, when the medium is favorable for the development of all these organisms, and when the temperature, oxygen supply and period of incubation are favorable. We can never obtain the ideal conditions but we can work out our technic so as to approach them as near as possible. The plate method has various limitations. The strict anaerobic microörganisms are excluded as well as the important groups of nitrifying, non-symbiotic nitrogen-fixing bacteria, sulfur-oxidizing bacteria, to some extent the denitrifying, symbiotic nitrogen-fixing, pectin- and cellulose-decomposing bacteria. Then, of course, the algae and protozoa are eliminated. A further limitation is the fact that high dilutions are necessarily used, so that if groups of microörganisms are determined on the plate, those organisms occuring only in small numbers will give a rather inaccurate count. However, the fact that various important groups of soil microörganisms do not develop on the plate does not detract very much from the value of the method since it holds true for all soils, the microörganisms that do develop on the plate are constant soil forms, and no one method, with the possible exception of the direct microscopic method, will allow a study of all soil microörganisms. Therefore, the plate method within defined limits, will serve as a certain measure of the quantitative bacterial flora. The soil microorganisms that develop on the plate include the fungi, the actinomycetes and those bacteria which are concerned in the decomposition of organic matter in the soil, assimilation and certain transformations of minerals as well as other not sufficiently studied activities.

The composition of the medium is one of the most important factors in the determination of numbers of microörganisms in the soil by the plate method. It must be of definite chemical composition (synthetic) and must allow the development of the greatest number of microörganisms.

Both gelatin and agar media are usually employed in the plate method. Gelatin was the first solid medium suggested by Koch for the study of pathogenic bacteria and was also used for the study of soil bacteria by the same investigator. The advantages of agar media over the gelatin are several, chief among which is the fact that agar can be kept at higher temperatures than gelatin, that the gelatin-liquefying microörganisms do not interfere with accurate counts, particularly after a long incubation period which allows all microörganisms to develop. Agar media can be prepared of an exact chemical composition. This cannot be said of gelatin which is in itself a nutrient for various microörganisms. For qualitative work, gelatin media no doubt present certain advantages as in the case of the separation of bacteria into liquefying and non-liquefying groups. But even this separation is of doubtful value and may give different results under different conditions, since, under the best of conditions, it is a qualitative rather than a quantitative distinction. In the case of actinomycetes, for example, we find that they nearly all liquefy the gelatin, some in 3–4 days and some in 40–50 days.

Similar disadvantages are found in the case of the nutrient agar used chiefly by the earlier soil bacteriologists. The introduction of media of exact chemical composition (Lipman and Brown, 9 and Fischer, 6) was an important step in the standardization of the plate method.

A comparative study of the various synthetic media to be used for the determination of numbers of soil microörganisms, has been given by Brown (2) and by Conn (4). The medium used in the present work is a modification of Brown's albumen agar:

K_2HPO_4	0.5 gm.
$MgSO_4$	0.2 gm.
Dextrose	10.0 gm.
Powdered egg-albumen	0.25 gm.
$Fe_2(SO_4)_3$	Trace
Agar	15.0 gm.
Distilled water	1000.0 cc.

All the ingredients, with the exception of the powdered egg-albumen are dissolved in boiling water, then filtered; the powdered egg-albumen is placed in a beaker and suspended by means of a stirring rod in a little distilled water, a drop of phenolphthalein is then added and sufficient 0.1 *N* NaOH is added from a burette until a distinct pink color is obtained. The egg-albumen is thus transformed into sodium albuminate, which is then added to the filtered agar and stirred in thoroughly. If the sodium albuminate solution has a few undissolved particles, it can be filtered first through a piece of filter paper. The use of sodium albuminate does away with the coagulation of albumen when added to the hot agar and allows the use of a standard product. The medium is tubed, in 10-cc. portions, and sterilized in the autoclave for 15 minutes at 15 lbs. pressure. The medium does not have to be freshly made up for each determination. The sterile tubed medium under cover for two months did not deteriorate so long as there was no appreciable drying out.

SOIL SAMPLING

It has been assumed that the same soil type, under the same conditions of treatment, contains at the same depth the same number of bacteria, and that soils differing in one way or another differ also in their bacterial activities (Hiltner and Störmer (8)). Due allowance must be made, however, to the natural variability of the soil itself. Where numerous samples are taken and thoroughly mixed, then carefully sampled, the danger is not so great as where only one or two samples are taken. By comparing the results obtained from various samples taken from the same uniform soil and treated alike, important variations have been obtained as pointed out in the previous paper of this series (19). For the actual field studies, four or five samples were taken from various parts of the plot, each sample being a composite of 3–4 borings, which were then thoroughly mixed.

Since the bacterial numbers are not the same at the various soil depths (18), the depth of sampling should be carefully considered. In taking samples for this study about ¼ inch of the surface was scraped away with a clean spatula and samples taken by means of a sampling tube to a depth of 6–6½ inches. The samples were taken into sterile containers, and counts made as soon as possible.

DILUTIONS AND PLATING

The first dilution was made as follows: 5 or 10 gm. or soil were added to a 250-cc. Erlenmeyer flask containing 100 cc. of sterile water; the flask was then shaken for exactly 5 minutes; this gave a dilution of 1:10 or 1:20. Of the first dilution 1 cc. was then taken out, without allowing the soil to settle, and transferred into a flask with 99 cc. of sterile water, giving dilutions of 1:1000 or 1:2000. Higher dilutions than the last were obtained by adding 1 or more cc. of the last dilutions to corresponding amounts of water with each new dilution. The final dilution was such as to allow 30–200 colonies to develop on the plate. The flask is shaken for about 30 seconds. One cc. of the final dilution is then transferred by means of fresh, sterile 1-cc. pipettes into Petri dishes and the cooled agar is added. Eight to ten plates were used for each count. The plates were then incubated at 25°C. for various periods of time and all the colonies were counted with the naked eye.

The importance of using a comparatively large amount of soil to make the first dilution and making that dilution comparatively low, like 1:10 or 1:20, has been recognized by the earlier bacteriologists (Remy '14) as well as in the more recent investigations (Northrup-Wyant '13), since a more representative soil sample is thus obtained. Too large an amount of soil, as used in some cases, to give too low a dilution like 1:2, is also objectionable since a thorough mixture of the soil and water is difficult. It is entirely possible to obtain a thorough suspension of bacteria in water, since it has already been demonstrated by Hiltner and Störmer (8) that, on sufficient shaking, all bacteria are washed off the soil particles which remain almost sterile.

There is small need of calling attention to the importance of not allowing the soil to stand in contact with the water for more than the few minutes necessary for the manipulations of shaking, diluting and plating out. A longer period will lead to an appreciable decrease in numbers, due to plasmolysis of microörganisms as pointed out by Hiltner and Störmer (8). An increase in numbers may be obtained only in special instances, as in the case of frozen soil, air-dry soil and subsoil.

INCUBATION AND COUNTS

Various periods of incubation were used in the preliminary experiments, these finally led to the adoption of a 7-days incubation at 25°C. A shorter period of incubation will not allow a full development of all microörganisms and a proper differentiation between the bacteria and actinomycetes.

The numbers of microörganisms were estimated in the preliminary experiments on the basis of soil dried in a electric oven at 100°C. to constant weight. However this method of calculating the data is hardly logical. Microörganisms usually decrease in number with a decrease in the water-content below the optimum. In this connection the author does not fully agree with the more recent workers (Northrup-Wyant, 13) in this field who calculated the bacterial numbers only on the basis of a dried soil. In a soil which is almost dry, the numbers, which are small at that, are increased only very little by figuring back to an air-dry basis. In the same soil at a much higher moisture content, it is found that the addition of moisture did not serve merely to dilute the soil, but had a stimulating effect upon the development of the bacteria, and it would hardly be advisable to multiply the numbers further to bring them to an arbitrary dry basis. The dry soil in itself does not signify anything, for the numbers depend on the relative moisture content as one of the important factors. Multiplying the numbers, to allow for the moisture, would be equivalent to doing the same calculation twice over.

To be scientifically accurate and have a basis for comparison, we should change the numbers in such a manner, by multiplying it by a moisture factor, so as to reduce those with a high moisture and increase those with a low moisture content. However, before such a factor has been found, the author agrees with Hiltner and Störmer (8) that the results should be reported per gram of moist soil (or even dry soil), giving also the moisture content of the particular soil as well as its optimum moisture (65–70 per cent of its water-holding capacity). The soil reaction, the author feels, should also be reported.

Perhaps, when our data are more complete, we may be able to calculate the potential bacterial activities from the soil type, its water content, reaction, nitrogen and carbon content, etc.

INFLUENCE OF MEDIUM

Preliminary work was carried out with the purpose of demonstrating the influence of the composition of the medium, reaction of medium, temperature of incubation, final dilution, etc., upon the numbers of microörganisms in the soil. The results are reported in tables 1–7.

Composition

Casein agar was prepared in a similar way to the egg-albumen agar except for replacing the egg-albumen by casein. Sodium asparaginate was made up according to directions given by Conn (4). Soil extract agar and urea nitrate agar were made according to directions given by Fred (7). The soil used for these preliminary studies was a greenhouse soil rich in organic matter, having an optimum moisture of 30 per cent and a reaction equivalent to pH 6.2. The fungi were not counted in these preliminary experiments, while under bacterial numbers, both bacteria and actinomycetes are included.

When the media of different composition are compared (table 1) the albumen agar, casein agar and soil extract agar are found to give the highest numbers. The last medium, although giving the largest numbers of all, has to be eliminated due to the fact that it is not standard in composition. The choice was then between the albumen and casein agar. The first was selected in spite of the fact that, in this experiment, it gave somewhat lower numbers than the casein agar. Albumen agar has been used by the writer for several years and has always given excellent results and stood out well in comparison with any other synthetic medium tested; it is also readily prepared and is of an exact chemical composition. Egg-albumen, of course, is not a pure protein, but since it is used in the powdered form, it is always readily duplicated. This medium was, therefore, selected for further work.[2]

TABLE 1

*Influence of composition of medium on bacterial numbers**

PLATE NUMBER	ALBUMEN AGAR	CASEIN AGAR	SOIL EXTRACT AGAR	SODIUM ASPARAGINATE AGAR	UREA NITRATE AGAR
	colonies	*colonies*	*colonies*	*colonies*	*colonies*
1	86	107	109	78	62
2	106	119	134	72	43
3	93	96	103	67	54
4	78	103	128	94	57
5	84	114	113	66	56
6	102	133	136	76	48
7	85	112	142	83	38
8	94	101	131	87	47
9	74	122	98	69	55
10	89	109	118	72	53
Mean	89.1 ±2.23	111.6 ±2.33	121.2 ±3.22	76.4 ±1.91	51.3 ±1.52
σ	10.44±1.57	10.97±1.65	15.10±2.27	8.97±1.35	7.16±1.08
C. V.	11.7 ±1.7 %	9.6 ±1.5 %	12.5 ±1.9 %	11.9 ±1.8 %	14.0 ±2.1 %
Em	2.5 %	2.1 %	2.6 %	2.5 %	2.96%

* Plates incubated at 25°C. for 7 days; the figures designate the number of all the colonies on the plate except the fungi. Dilution 1:200,000.

Temperature and period of incubation

The data presented in table 2 show the influence of temperature and period of incubation upon the bacterial numbers found in a soil. When the plates are incubated at room temperature, practically no colonies developed in 2 days, while in 12 days not all the colonies seemed to have developed as yet, since more than twice as many colonies have been found in 12 days than in 5 days. By incubating the plates at 37°, not all the microörganisms are found to develop into colonies and the plates dry up on prolonged incubation. A temperature of 25 to 27°C. proved to be the most favorable, one or two degrees either way having little influence with a long period of incubation. The plates should certainly be incubated longer than even 5 days. Further experiments along this line have shown that, at 25°, there is very little increase in numbers

---

[2] After this study was completed, the author in coöperation with Dr. Fred of the University of Wisconsin (20) suggested, as definite uniform media for the determination of total numbers of microörganisms in the soil, a modification of the albumen agar given above and casein agar. The modification of the albumen agar consists in reducing the amount of dextrose from 10 to 1 gm. per liter, so as to prevent the development of spreading colonies. The casein agar is the same as the albumen agar, only 1 gm. of purified casein dissolved in 8 cc. of 0.1 *N* NaOH is substituted for the egg-albumen. However, the albumen agar used in the studies reported in this as well as in the following paper, was of the composition reported in the text above.

TABLE 2

*Influence of temperature and period of incubation on bacterial numbers**

PLATE NUMBER	ROOM TEMPERATURE			25°C.			37°C.		
	Incubation			Incubation			Incubation		
	2 days	5 days	12 days	2 days	5 days	12 days	2 days	5 days	12 days
	colonies	*colonies*	*colonies*	*colonies*	*colonies*	*colonies*	*colonies*	*colonies*	*colonies*
1	No growth	76	152	38	120	164	28	102	Plates dried up
2		70	169	42	84	158	24	123	
3		58	128	35	105	172	16	126	
4		78	125	47	139	191	31	104	
5		56	130	48	118	197	26	134	
6		73	143	44	128	183	26	92	
7		53	88	43	126	152	21	79	
8		61	154	56	142	168	24	83	
9		64	134	39	81	179	18	67	
10		48	164	40	116	161	25	76	
Mean		63.7 ±2.18	138.7 ±6.0	43.2 ±1.28	115.9 ±4.39	172.5 ±3.14	23.9 ±0.9	98.6 ±4.93	
σ		10.22±2.28	23.47±3.55	6.01±1.34	20.65 ±3.11	14.72±2.21	4.26±0.95	23.14±5.17	
C. V.		16.0 ±3.6 %	17.6 ±2.5 %	13.9 ±3.1 %	17.8 ±2.7 %	8.5 %	17.8 ±4.0 %	23.5 ±5.2 %	
Em		3.42%	3.6 %	2.96%	3.79%	1.82%	3.77%	5.0 %	

* The numbers represent all the colonies on the plate, except the fungi. Dilution 1:100,000.

above 7 days, so that this period has been decided upon for future work. In this connection, attention should be called to the fact that Conn also found a seven-day period, at a temperature of 25°, sufficient since he seldom found any appreciable increase on further incubation.

The futility of short incubation periods (2–3 days), sometimes even at room temperature, is thereby made clear.

Reaction of medium

A slight acidity (+0.5—+0.25) has usually been recommended as the optimum reaction of the medium used for the bacteriological analysis of soils. Several portions of egg-albumen agar were adjusted to definite hydrogen-ion concentrations by means of 1.0*N* NaOH and 1.0*N* H_2SO_4 solutions and used for the plating out of bacterial numbers.

TABLE 3

*Influence of reaction of medium upon the growth of bacteria on the plate**

PLATE NUMBER	pH = 5.2	pH = 6.4	pH = 6.8	pH = 7.2	pH = 7.6
	colonies	*colonies*	*colonies*	*colonies*	*colonies*
1	62	77	63	53	44
2	56	105	66	66	42
3	52	83	91	41	38
4	89	69	79	64	34
5	86	76	87	56	41
6	47	97	83	45	43
7	96	92	88	58	45
8	51	89	97	51	39
9	57	86	61	44	24
10	69	78	76	48	36
Mean	66.5 ±3.76	85.2 ±2.31	79.1 ±2.64	52.6 ±1.83	38.6 ±1.32
σ	17.68±2.66	10.87±1.64	12.41±1.87	8.6 ±1.29	6.22±0.94
C. V.	26.6 ±4.0 %	12.7 ±1.9 %	15.7 ±2.4 %	16.3 ±2.4 %	16.1 ±2.4 %
Em	5.65%	2.7 %	3.34%	3.5 %	3.4 %

* Dilution 1:200,000. Plates incubated 12 days at 25°. All colonies, exclusive of fungi, are reported.

The data presented in table 3 point definitely to the fact that a reaction of the medium equivalent to an hydrogen-ion concentration of about pH 6.4 is best. With more acid media, there is a decrease in bacterial numbers accompanied by a greater overgrowth of fungi. When the medium is made less acid there is also a drop in numbers, particularly above the neutral point, so that, at a pH 7.6, there are already less than a half as many colonies of bacteria developing than at pH 6.4. A reaction of about pH 6.5 is therefore, best. This happens to be the reaction of the egg-albumen agar when prepared according to the directions given above.

Method of preparing the dilutions

It seems to be generally agreed that the number of colonies to be allowed per plate should be between 30 and 200, for agar plates (Breed and Dotherrer, 1), or a narrower limit 50 to 150 for gelatin plates (Conn, 4). However, where the incubation is at 25° for 7 days, there is danger of fungi overgrowing the plates. A plate badly overgrown with fungi, particularly in the case of certain *Mucorales*, should be discarded and not considered in the final count. With the medium used, there is very little danger of bacterial spreaders.

The next two experiments deal with the method of preparing and time of shaking the soil with water in preparing the first dilution (table 4) and influence of the final dilution (table 5).

TABLE 4

Influence of stirring and time of shaking upon bacterial numbers[*]

PLATE NUMBER	SOIL STIRRED IN MORTAR	SHAKEN 1 MINUTE	SHAKEN 5 MINUTES	SHAKEN 10 MINUTES
	colonies	*colonies*	*colonies*	*colonies*
1	144	104	158	144
2	168	114	168	168
3	170	125	162	171
4	153	136	184	155
5	156	113	162	147
6	178	122	181	158
7	149	131	151	175
8	153	116	174	168
9	171	119	189	167
10	158	108	179	151
Mean	160.0 ±2.3	118.8 ±2.11	170.8 ±2.65	160.4 ±2.31
σ	11.07±2.70	9.91±2.22	12.49±1.88	10.83±2.42
C. V.	6.9%	8.3%	7.3%	6.8%
Em	1.4%	1.77%	1.55%	1.44%

[*] Plates incubated 7 days at 25°C. Dilution 1:100,000. All colonies except fungi are reported.

The stirring referred to in the first column of table 4 was done by thoroughly stirring 5 gm. of soil in sterile water, in a sterile mortar for 5 minutes, pouring off the supernatant liquid into a sterile flask, stirring residue again for 2–3 minutes with a fresh portion of sterile water, and so forth, until only a few grains of pure sand remained. The shaking referred to in the other three columns was done by shaking 5 gm. of soil in 100 cc. of sterile water and withdrawing samples after 1, 5, and 10 minutes for the further dilutions. The final dilution for this experiment was 1:100,000. The results reported in table 5 were obtained by shaking the original dilution 5 minutes, and making the final dilutions 1 to 20,000, 50,000, 100,000, 200,000, 500,000 and 1,000,000.

The results obtained in table 4 justify, without further discussion, the conclusion that 5 minutes shaking is sufficient for suspending all the bacteria in

TABLE 5

*Influence of final dilution upon the bacterial numbers**

PLATE NUMBER	DILUTION, 1:20,000		DILUTION, 1:50,000	
	Incubation		Incubation	
	3 days	7 days	3 days	7 days
1	192	Too numerous to count	127	156
2	238		124	146
3	262		148	172
4	206		143	179
5	227		108	124
6	231		113	138
7	214		137	149
8	199		132	167
9	223		141	153
10	211		117	133
Mean	220.3 ±4.10		129.0 ±2.88	152.7 ±3.73
σ	19.25±2.93		13.51±2.03	17.53±2.64
C.V.	8.7 %		10.5 ±1.6 %	11.5 ±1.7 %
Em	1.86%		2.23%	2.44%
Average number of Bacteria per gm. of wet soil	4,403,000		6,450,000	7,635,000

PLATE NUMBER	DILUTION, 1:100,000		DILUTION, 1:200,000	
	Incubation		Incubation	
	3 days	7 days	3 days	7 days
1	76	113	29	59
2	65	106	28	62
3	56	103	30	61
4	74	112	27	69
5	62	142	38	73
6	59	135	24	64
7	67	129	33	68
8	51	127	31	56
9	63	118	41	61
10	69	131	36	74
Mean	64.2 ±1.64	121.6 ±2.81	31.7 ±1.12	64.7 ±1.28
σ	7.76±1.17	13.18±1.99	5.29±0.79	6.04±0.91
C. V.	12.1 ±1.8 %	10.8 ±1.5 %	16.6 ±2.5%	9.3 %
Em	2.55%	2.31%	3.53%	2.0 %
Average number of Bacteria per gm. of wet soil	6,420,000	12,160,000	6,340,000	12,940,000

* Numbers of all microörganisms, except fungi, are given.

TABLE 5—*Continued*

PLATE NUMBER	DILUTION, 1 500,000		DILUTION, 1·1,000,000	
	Incubation		Incubation	
	3 days	7 days	3 days	7 days
1	14	18	4	11
2	19	26	4	17
3	18	22	9	19
4	13	21	5	13
5	13	19	5	14
6	13	18	6	17
7	17	23	4	11
8	16	21	7	13
9	17	25	5	9
10	21	29	9	12
Mean	16 1 ±0 60	22 2 ±0 77	5 8 ±0 41	12 6 ±0 76
σ	2 81±0 42	3 62±0 55	1 93±0 29	3 53±0 53
C. V.	17 4 ±2 6 %	16 3 ±2 5 %	33 3 ±5 0%	28 0 ±4 2 %
Em	3 73%	3 47%	7 07%	6 03%
Average number of Bacteria per gm. of wet soil	8,050,000	11,100,000	5,800,000	12,600,000

the water. One minute is insufficient, while a period greater than 5 minutes proves to be injurious.

When the various dilutions are compared, it is found that both too low dilutions and too high dilutions give unfavorable results. With the low dilutions too many colonies develop on the plates and it is impossible to determine accurately even the number of colonies that have developed. With too many colonies on the plate, many microörganisms, particularly those that develop only late, fail to develop at all.

It is interesting to note that, while at 1:20,000 dilution the colonies were, at 7 days, so numerous that no accurate count could be made, particularly due to overgrowth of fungi and that with the 1:50,000 dilution, an accurate count was made, but the numbers of organisms obtained are much less than with the higher dilutions. This simply indicates that, with too many colonies on the plate, many organisms simply fail to develop. Another disadvantage of the too low dilutions is the fact that it is difficult to make an accurate differentiation, under these conditions, between bacterial and actinomyces colonies. Of course the advantage of the low dilution lies in the smaller error obtained, but this can be obviated by the use of a larger number of plates for the count.

In the case of too high dilutions, like those of 1:500,000 and 1:1,000,000, there is apt to be not only greater variability with a greater error involved and the actual elimination of many specific types but the actual count may be smaller.

In the case of the two highest dilutions used in this experiment, the number of colonies developing on the plate was below 30 and although in this case there was plenty of room for development, the count was less, even with a 7-days incubation period, than with the optimum dilution (1:100,000 and 1:200,000). This, chiefly, is the reason why the number of colonies allowed per plate has been usually recommended as between 40 and 200. The optimum dilution for ordinary field soils is from 1:100,000 to 1:200,000. For poor sandy soils, a lower dilution may have to be used; for heavily manured soils or green-house soils, higher dilutions should be used.

The results reported here are directly opposed to the claim of Rossi (15) that the number of microörganisms present in the soil depends entirely on the dilution and increases with the higher dilutions. That is true only within certain narrow limits (below the optimum dilution).

TABLE 6

*The use of tap water and salt solution (0.85 per cent NaCl) as diluents for making bacterial counts**

PLATE NUMBER	TAP WATER		SALT SOLUTION	
	All colonies except fungi	Actinomycetes	All colonies except fungi	Actinomycetes
	colonies	*colonies*	*colonies*	*colonies*
1	91	24	71	22
2	83	20	72	23
3	68	22	76	26
4	62	19	64	19
5	71	21	59	26
6	79	18	71	16
7	86	26	56	21
8	78	21	61	22
9	85	27	62	16
10	82	23	76	23
Mean	78.5 ±1.91	22.1 ±0.62	66.7 ±1.54	21.4 ±0.75
σ	8.98 ±1.35	2.92±0.63	7.25±1.09	2.53±0.53
C. V.	11.4 ±1.7	13.2 ±1.9 %	10.8 ±1.6 %	16.5 ±2.5
Em	2.43%	2.81%	2.31%	2.5%

* Dilution 1:200,000. Plates incubated 7 days at 25°C.

DILUENT

Ordinary sterile tap water is commonly used in making dilutions. The use of bouillon or sugar solution has not been found beneficial, while a solution of 0.4 per cent NaCl and 0.4 per cent KCl has actually been found injurious (Hiltner and Stormer, 8). Ordinary sterile tap water was compared with saline (0.85 per cent NaCl) solution with the results presented in table 6.

No advantage is obtained from the use of salt solution over ordinary tap water. If anything, there is an injurious effect due to the use of the salt solution, confirming the results of Hiltner and Störmer, who used a mixture of sodium and potassium chlorides. No appreciable difference has been found in the number of actinomycetes with both diluents. The use of distilled water for making dilutions should be condemned, since plasmolysis will readily take place.

SUMMARY

With these preliminary experiments in mind, we can now establish some of the important points to be observed in the determination of bacterial numbers in the soil.

1. A medium of standard composition should be used, containing no peptone, meat extract, soil extract or similar material, which would vary greatly in composition. In addition to the necessary minerals and carbohydrate, the medium should contain a definite nitrogen source, like asparagine, purified casein or powdered egg-albumen.
2. The final reaction of the medium should be about pH 6.2–6.8, with an optimum at pH 6.5.
3. Sterile tap water should be used for making the dilution.
4. The soil should be shaken uniformly, for 5 minutes, in preparing the first dilution.
5. The original dilution should be 1:20 or 1:10, high enough to give a ready distribution of the bacteria, and low enough to allow a representative sample to be taken. The further dilutions should be uniform, preferably 1:10 or 1:100. The final dilution should be made in such a manner, as to give 40–200 colonies of microörganisms excluding fungi, per plate.

Where a count of soil fungi is wanted, special acid media should be used having a pH 4.0 (like raisin agar and special synthetic agar (Waksman, 19), which due to their nature, do not allow any development of actinomycetes or bacteria, so that a low dilution (one fiftieth to one two-hundredth of that used for bacteria) can be used. This, combined with a short incubation period will allow a count of fungi, involving a comparatively low probable error.

6. At least 3-5 samples, composite if possible, should be taken from each soil examined for each determination.
7. At least 6 10 plates should be used in plating out each sample. These last two points are important where we want to work out the variability of numbers of microörganisms, and reduce these to a mathematical standard.
8. The plates should be incubated at 25°C. for at least 7 days or at room temperature for at least 14 days, the first to be preferred due to uniform temperature.
9. Plates badly overgrown with fungi, particularly in case of certain *Mucorales*, should be discarded from the counts.
10. The numbers should be computed on the basis of wet soil or soil dried to constant weight, in each case stating the moisture-content and the moisture-holding capacity, or optimum moisture, of the particular soil.
11. The most probable error of the counts should not be greater than 2.0 to 2.5 per cent for each soil, and not above 3.0 per cent for each soil sample.

REFERENCES

(1) BREED, R. S., AND DOTTERRER, W. D. 1916 The number of colonies allowable on satisfactory agar plates. N. Y. (Geneva) Agr. Exp. Sta. Tech. Bul. 53, p. 3–11.

(2) BROWN, P. E. 1913 Methods for bacteriological examination of soils. Iowa Agr. Exp. Sta., Res. Bul. 11, p. 381–407.

(3) CHESTER, F. D. 1904 Bacteriological analysis of soils. Del. Agr. Exp. Sta. Bul. 65, p. 51–76.

(4) CONN, H. J. 1914 Culture media for use in the plate method of counting soil bacteria. N. Y. (Geneva) Agr. Exp. Sta. Tech. Bul. 38, p. 1–34.

(5) CONN, H. J. 1918 The microscopic study of bacteria and fungi in soil. N. Y. (Geneva) Agr. Exp. Sta., Tech. Bul. 64, 1–20.

(6) FISCHER, H. 1910 Zur Methodik der Bakterienzählung. *In* Centbl. Bakt. (etc.), Abt. 2, v. 25, p. 457–59.

(7) FRED, E. B. 1916 Laboratory Manual of Soil Bacteriology, p. 94–96. W. B. Saunders, Philadelphia.

(8) HILTNER, L., AND STÖRMER, K. 1903 Studien über die Bakterienflora des Ackerbodens, mit besonderer Berücksichtigung ihres Verhaltens nach einer Behandlung mit Schwefelkohlenstoff und nach Brache. *In* Arb. K. Gsndhtsamt., Biol. Abt., v. 3, p. 445–54.

(9) LIPMAN, J. G., AND BROWN, P. E. 1910 Media for the quantitative estimation of soil bacteria. *In* Centbl. Bakt. (etc.), Abt. 2, v. 25, p. 447–454.

(10) LÖHNIS, F. 1904 Bodenuntersuchung. Zur Methodik der bakteriologischen Bodenuntersuchung. *In* Centbl. Bakt. (etc.), Abt. 2, v. 12, p. 262; v. 14, p. 1–9; v. 17, p. 518–528.

(11) LÖHNIS, F. 1910 Handbuch der landwirtschaftlichen Bakteriologie. Borntraeger, Berlin.

(12) LÖHNIS, F. 1912 Ziele und Wege der bakteriologischen Bodenforschung. *In* Landw. Jahrb., v. 42, p. 751–765.

(13) NORTHRUP-WYANT, Z. 1921 A comparison of the technic recommended by various authors for quantitative bacteriological analysis of soil. *In* Soil Sci., v. 11, p. 295–303.

(14) REMY, TH. 1902 Bodenbakteriologische Studien. *In* Centbl. Bakt. (etc.), Abt. 2, v. 8, p. 657–662, 699–705, 728–735, 761–769.

(15) ROSSI, GIACOMO 1921 Preliminary note on the microbiology of the soil and the possible existence therein of invisible germs. *In* Soil Sci., v. 12, p. 409–412.

(16) RUSSELL, E. J., AND APPLEYARD, A. 1917 The influence of soil conditions on the decomposition of organic matter in the soil. *In* Jour. Agr. Sci., v. 8, p. 385–417.

(17) VOORHEES, E. B., AND LIPMAN, J. G. 1907 A review of investigations in soil bacteriology. U. S. Dept. Agr. Office Exp. Stas. Bul. 194.

(18) WAKSMAN, S. A. 1916 Bacterial numbers in soils, at different depths, and in different seasons of the years. *In* Soil Sci. v. 1, p. 363–380.

(19) WAKSMAN, S. A. 1922 Microbiological analysis of soil as an index of soil fertility: I. The mathematical interpretation of numbers of microörganisms in the soil. *In* Soil Sci., v. 14, p. 81–101.

(20) WAKSMAN, S. A., AND FRED, E. B. 1922 A tentative outline of the plate method for determining the number of microörganisms in the soil. *In* Soil Sci., v. 14, p. 27.

NITRATE ACCUMULATION UNDER STRAW MULCH

W. A. ALBRECHT

Missouri Agricultural Experiment Station

Received for publication January 14, 1922

The surface application of straw has come into use quite widely by vegetable gardeners, orchardists, wheat growers, and others. Potatoes are often grown under a heavy mulch of four to eight inches of straw, and it is a general idea that mulching with wheat straw is good agricultural practice. Noticeable detrimental effects have suggested shortage of nitrogen so that along with other nitrate studies the writer has given attention to the effect of the straw mulch on nitrate production.

HISTORICAL

Previous to 1921 no study of this kind was found reported, but only recently there were reported results from a similar study by Scott (4) where the surface applications of straw on wheat as low as 2 tons per acre had depressive effects on the nitrate accumulation in the soil.

Laboratory studies have been common (1, 5, 7), showing detrimental effects on nitrification by organic matter in solution, but the mixture of organic materials through the soil (3) has not been so destructive.[1] The effect of the straw mulch as a surface dressing on nitrate accumulation remained to be tested.

EXPERIMENTAL

The following study was conducted from 1917 to 1919 inclusive, on a brown silt loam of glacial origin with fine friable structure to a depth of about 8 inches. Below this point it grades quickly into a rather tight, brownish mottled, silty, clay loam. The surface sloped eastward with a 3-per cent grade and the plots were surrounded by board curbing to prevent cross washing, so that the plots served as independent though adjacent units. Four plots, 1/80 acre each, were treated as follows:

PLOT NUMBER	SPRING TREATMENT	SUMMER TREATMENT
3	Plowed	Fallow, Straw mulch 8 tons per acre, weeds pulled
4	Plowed	Fallow, surface scraped
5	Unplowed	Fallow, surface scraped
7	Plowed	Fallow, surface cultivated

---

[1] Confirmed by Dr. J. G. Lipman, N. J. Agr. Exp. Sta., in recent correspondence.

The spring plowing was eight inches deep and the surface scraping of plots 4 and 5 was done by means of a hoe to cut off small weeds. The hoe was used to cultivate plot 7 to a depth of 2–3 inches after rains. The straw mulch was applied at the rate of 8 tons after plowing. In the following spring the remaining straw was removed, the ground plowed as soon as dry enough, and remulched with fresh wheat straw. Few weeds came through the mulch and little care was needed to keep them down. At the start, the land was cleared of a thin wheat crop and uniform conditions for all plots were represented.

Samples of the surface 7 inches of soil were taken every three weeks except when the soil was frozen, which occurred only in the winter of 1917–18. The open winters of the remaining two years caused little irregularity in this respect. Nine samples per plot were drawn in schematic order to form a composite sample. Only the surface soil data are reported because of the nature of the subsoil in this case and the lack of differences in nitrates at greater depth as shown by sub-surface samplings.

As soon as possible after sampling, the soil was mixed, 100-gm. portions taken, dried at 108°C. for 8 to 10 hours, and loss of moisture determined. These were then extracted with 300 cc. of 1/16 *N* hydrochloric acid. The nitrates were determined in 200 cc. aliquots by boiling down, reducing with DeVarda's metal, and distilling into standard sulfuric acid. Calculations are given in pounds of nitrogen as nitrate per 2,000,000 pounds of oven dried soil.

Table 1 gives the data on nitrate nitrogen in the plots during the time studied, while figures 1 and 2 give these data graphically.

From the data and graphs it is evident that the straw mulch prohibits the accumulation of nitrates in the soil. During the three years of study the nitrate nitrogen under mulch was never greater than 27 pounds per two million of soil,—reached in late July of the excessively hot and dry summer of 1918,—while in plot 4, without the mulch, this figure went over 200. Comparatively few of the minimum determinations of the unmulched plots went below even the maximum on the mulched plot, and the graph of the mulched plot meets that of any other plot for only two dates during the three years.

As to the cause of this failure of nitrates to accumulate, one must consider the moisture content of the soil. Table 2 gives the nitrates of plot 3 together with the percentage moisture at the corresponding samplings. Figure 3 shows this relation graphically. From the curves it is evident that there is a close reciprocal relation of moisture to nitrates. This relation points out that moisture was an inhibiting factor, for only as the moisture was lessened did nitrates accumulate. Evidently moisture is the main cause, directly or indirectly, for the inhibition of nitrate accumulation. The mulch serves to increase the moisture in the soil by increasing absorption through lessened run off and also by preventing evaporation.

One might be led to believe that nitrates might be formed but removed by the excessive water caught by the mulch. Were such true, the production of

TABLE 1

Nitrate nitrogen in mulched soil as compared with unmulched

(In 2,000,000 lbs. of soil)

1917					1918					1919				
Date sampled	3*	4	5	7	Date sampled	3	4	5	7	Date sampled	3	4	5	7
	lbs.	*lbs.*	*lbs.*	*lbs.*		*lbs.*	*lbs.*	*lbs.*	*lbs*		*lbs.*	*lbs.*	*lbs.*	*lbs.*
May 12	9	13	21	14	Mar. 1	13	155	80	76	Jan. 27	8	95	39	24
June 4	0	26	29	24	Mar. 22	19	158	86	86	Feb. 19	10	90	79	27
June 26	5	62	46	41	Apr. 12	17	177	89	80	Mar. 12	8	96	63	15
July 19	14	106	79	61	May 3	11	63	26	21	Apr. 4	12	119	118	38
Aug. 9	9	97	98	75	May 24	5	82	47	25	Apr. 25	14	115	105	35
Aug. 30	7	103	88	54	June 14	24	208	104	82	May 23	5	118	100	18
Sept. 22	14	171	124	88	July 5	16	131	85	74	June 7	12	111	89	9
Oct. 13	10	189	125	65	July 26	27	124	29	31	July 2	8	98	107	
Nov. 3	5	194	140	84	Aug. 16	20	166	94	78	July 24	12	159	204	42
Nov. 24	17	205	171	104	Sept. 6	12	157	63	23	Aug. 8	16	81	265	43
					Sept. 27	9	238	126	76	Sept. 1	8	174	178	36
					Oct. 19	20	72	111	64	Sept. 22	13	146	213	24
					Nov. 8	7	126	167	55	Oct. 13	13	143	253	22
					Nov. 29	12	114	70	27	Nov. 3	10	112	177	17
					Dec. 21	9	98	60	17	Nov. 21	17	89	241	21

* Plot 3. Straw mulch.
Plot 4. Spring plowed; scraped.
Plot 5. Unplowed; scraped.
Plot 7. Spring plowed: cultivated.

TABLE 2

Nitrate nitrogen and per cent moisture under mulched soil (Plot 3)

1917			1918			1919		
Date	Nitrate	Moist-ure	Date	Nitrate	Moist-ure	Date	Nitrate	Moist-ure
	*lbs.**	*per cent*†		*lbs.*	*per cent*		*lbs.*	*per cent*
May 12	9	20.6	Mar. 1	13	22.5	Jan. 27	8	23.3
June 4	0	25.2	Mar. 22	19	23.4	Feb. 19	10	23.4
June 26	5	23.9	Apr. 12	18	22.2	Mar. 12	8	23.2
July 19	14	21.3	May 3	11	23.7	Apr. 4	12	22.8
Aug. 9	9	22.7	May 24	5	25.0	Apr. 25	14	20.1
Aug. 30	7	22.7	June 14	24	20.5	May 23	5	24.7
Sept. 22	14	20.0	July 5	16	22.4	June 7	12	24.1
Oct. 13	10	21.4	July 26	27	18.2	July 2	8	
Nov. 3	5	21.8	Aug. 16	20	20.9	July 24	12	20.1
Nov. 24	17	19.0	Sept. 6	12	23.5	Aug. 8	16	20.6
			Sept. 27	9	22.7	Sept. 1	8	22.3
			Oct. 19	20	25.0	Sept. 22	13	24.1
			Nov. .8	7	24.8	Oct. 13	13	22.9
			Nov. 29	12	24.7	Nov. 3	10	19.1
			Dec. 21	8	24.4	Nov. 21	17	14.2

* Nitrate nitrogen is given as pounds per two million soil.

† Moisture is expressed as per cent of oven-dry soil.

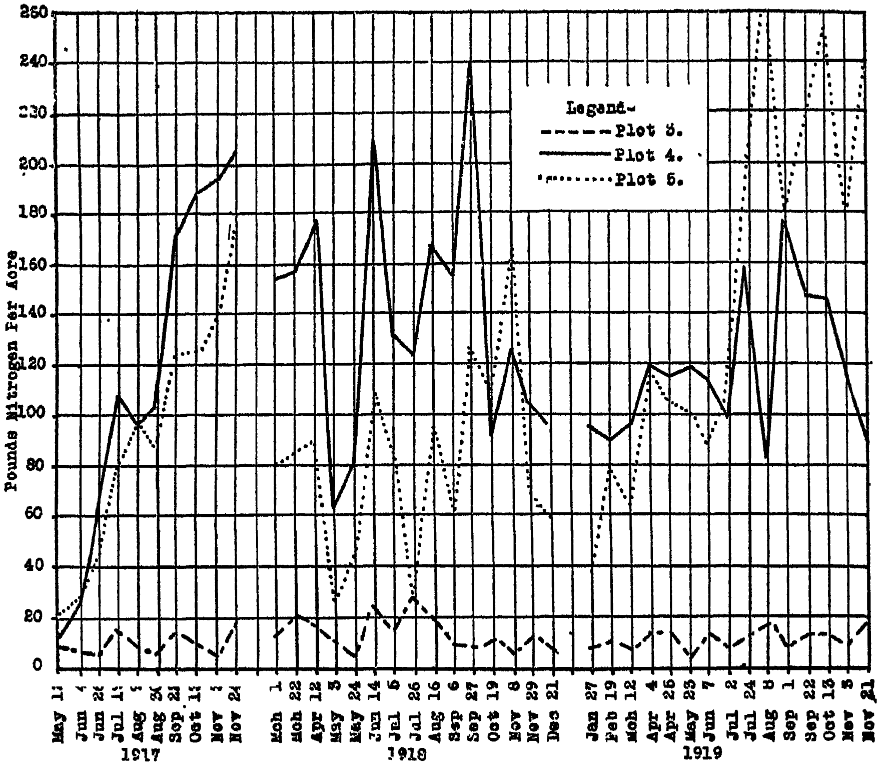


FIG. 1. NITRATE-NITROGEN IN SOIL UNDER MULCH (PLOT 3) AS COMPARED WITH UNMULCHED SOILS (PLOT 4, SPRING PLOWED AND SCRAPED; PLOT 5, UNPLOWED AND SCRAPED)

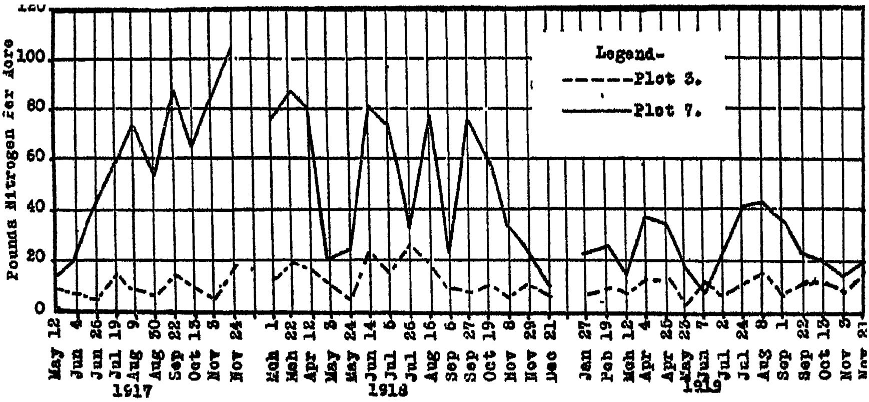


FIG. 2. NITRATE-NITROGEN IN SOIL UNDER MULCH (PLOT 3) AS COMPARED WITH UNMULCHED SOIL (PLOT 7,—SPRING PLOWED AND CULTIVATED THREE SEASONS)

nitrates after rain should give wider fluctuations than 19 pounds of nitrogen, which was the maximum increase found during any 3-week period of no rainfall following much rain. During the corresponding time, plots 4, 5, and 7 (with no mulch) showed increases of 126, 67, and 47 pounds respectively. Examination of any other 3-weeks period with no rainfall to remove the nitrate fails to show significant increase in nitrate content under the mulch, while in the other plots it shows great increases and establishes the certainty that nitrates are not accumulating at a rate comparable to that in soils without mulch. Had significant nitrate accumulation gone on in the surface and had the nitrates been carried downward, these could have been detected in the subsurface of heavy clay soil. Determination of nitrates on five inches of soil below the surface layer showed nitrates in constant amounts too insignificant to indicate sudden nitrate movement or important disturbances. No more than 12 pounds of nitrogen per 2,000,000 of soil were ever found in this layer.

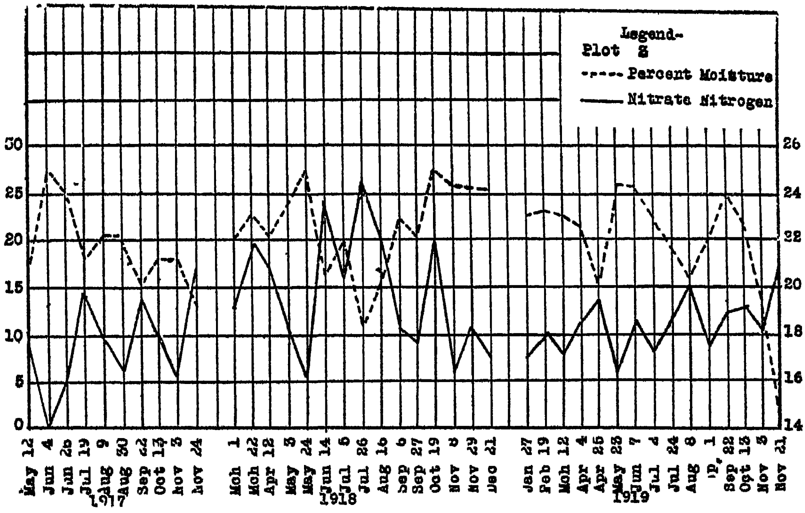


Fig. 3. Comparison of Nitrate-Nitrogen with Percentage Moisture in Soil under Mulch (Plot 3)

According to Gainey and Metzler (2), the optimum moisture for nitrate production in a soil with any degree of compactness, will be reached when it contains approximately two-thirds of the total amount of moisture it will retain. Tests on this soil by the Hilgard cup method gave 40.23 as its moisture-holding capacity and according to the above, the optimum should be 26.82 per cent. Instead, there was no accumulation at 24–25 per cent, which was the maximum water content found in this soil. This suggests that possibly Gainey's figure is too high or the moisture is not the sole, direct, contributing cause of the failure of nitrates to accumulate. For that reason daily temperature records were taken at a depth of 3 inches below the surface of the soil on the mulched plot, the unplowed plots and the plowed-scraped plot during the summer of 1918.

During June, July and August the average temperature of the mulched soil at 5.30 p.m. was 25.35°C. while on the plowed-scraped soil it was 33.06°C. and in the unplowed 33.92°C. The maxima were 30°, 40° and 39.5°C. in the order given above, showing that the mulched soil failed by ten degrees centigrade of being as warm as the plots not mulched. However, a temperature of 25.35°C. (the average for plot 3) should be high enough to encourage significant bacterial action so that higher nitrate production could be expected were temperature the sole limiting factor.

The lower temperature, of course, is due to the moisture of which the mulched soil always contained large amounts, and only in dry seasons did it dry out enough to put the soil in good tilth, or at about 50 per cent of moisture-holding capacity. By using this moisture figure for tilth rather than Gainey's for nitrification, the optimum for this soil would be 20.1 per cent or considerably below 22.4, the average water content of all the samplings for this soil. At this rate the soil was wetter than optimum by 2.3 per cent during most of the time under study and moisture may be closely connected in causal relation with the suppression of nitrate accumulation.

This inhibition of nitrate accumulation by the mulch is of particular significance to the vegetable gardner, horticulturist, wheat grower and others who have been using the straw mulch. Since crops require nitrogen and use it to best advantage in the nitrate form, it is possible that the low concentration of nitrates present at any time under the straw may be an inhibiting factor to best growth of certain crops. Since wheat is a heavy nitrate feeder it is readily possible that some of the detrimental results from mulching wheat have come from this cause as reported by Scott (4) even with as light an application as 2 tons per acre. Mulching with straw raises the moisture supply, but evidently does not allow nitrate accumulation to the concentration to be expected. Whether the process of nitrification goes on regularly and the nitrates are removed or changed by some other biological process encouraged through the presence of soluble organic matter,—as is the opinion of Lipman[1]—is a question which can be answered only by further study and analytical work.

Results obtained in this study suggest that mulching with straw keeps down the concentration of nitrates in the soil, and one may readily expect only those crops to do well which are able to obtain their nitrogen from this low a concentration. The nitrate content of soils under mulch is far enough below those under crops in similar studies at Missouri, or reported by Whiting (6), to suggest that mulching with straw is a practice to be used with care and discretion rather than one of universal application.

---

[1] Confirmed by Dr. J. G. Lipman, N. J. Agr. Exp. Sta., in recent correspondence.

REFERENCES

(1) Coleman, L. C. 1908 Untersuchungen über Nitrifikation. Centbl. Bakt., Abt. 2, v. 20, p. 401.

(2) Gainey, P. L., and Metzler, L. F. 1917 Some factors affecting nitrate-nitrogen accumulation in soil. *In* Jour. Agr. Res., v. 11, p. 43–46.

(3) Lyon, T. L., and Bizzell, J. A. 1913 Some relations of higher plants to the formation of nitrates in the soil. N. Y. (Cornell) Agr. Exp. Sta. Mem. 1.

(4) Scott, Herschel 1921 The influence of wheat straw on the accumulation of nitrates in the soil. *In* Jour. Amer. Soc. Agr., v. 13, p. 233–258.

(5) Stevens, F. L., and Withers, W. A. 1910 The inhibition of nitrification by organic matter compared in soils and in solutions. *In* Centbl. Bact. Abt. 2, v. 27, p. 169.

(6) Whiting, A. L., and Schoonover, W. R. 1920 Nitrate production in field soils in Illinois. Ill. Agr. Exp. Sta. Bul. 225.

(7) Winogradsky, S., and Omeliansky, V. 1899. Über den Einflusz der organischen Substanzen auf die Arbeit der Nitrifixierenden Mikroben. *In* Centbl. Bact., Abt. 2., v. 5, p. 329.

SULFUR OXIDATION IN INOCULATED AND UNINOCULATED GREENSAND MIXTURES AND ITS RELATION TO THE AVAILABILITY OF POTASSIUM[1]

W. RUDOLFS[2]

New Jersey Agricultural Experiment Stations

Received for publication October 18, 1921

Although a study of the literature shows that the application of greensand for agricultural purposes has proved beneficial, and although good results from the use of greensand were recognised long ago in England and France and later in America, especially in New Jersey, farmers did not turn to these great resources even during the war-time scarcity of potassium. This is undoubtedly due to the fact that all greensands found in America have but a low percentage of potassium and to the ease with which the farmer in ordinary times can buy readily available potash.

The potassium of the greensand is but slowly available for plant food and the addition of greensand to a soil serves mainly the future crops. For building up a soil, poor in potassium, it has proven to be a great asset. The value of greensand as a fertilizer has been, and is still often ascribed to the calcium carbonate and phosphorus present. Since a large number of the New Jersey greensands contain from 1 to 3 per cent phosphoric acid and from 3 to 7 per cent potassium, it is probable that the immediately beneficial effects can be attributed to both these constituents. In many cases not much calcium carbonate is present and the greensand dug often has a decided acid reaction. The greensands and greensand marls found in England often contain as much as from 8 to 10 per cent of K_2O, but usually little or no phosphoric acid.

REVIEW OF LITERATURE

The war stimulated the study of practical methods for rendering the potassium soluble Experiments were conducted in several states upon the availability of greensands for plant food, and upon the possibilities of securing means to treat the greensand. The Eastern Potash Corporation in New Jersey (13) claims to have found a factory method which consists of treating the greensand with lime and steam under pressure, for which process a plant is under construction.

---

[1] Paper No. 92 of the Journal series New Jersey Agricultural Experiment Stations, Department of Soil Chemistry and Bacteriology. This paper will appear in RUTGERS COLLEGE STUDIES, vol. 1.

[2] Part of a thesis submitted to the faculty of Rutgers College and the State University of New Jersey in partial fulfillment for the requirements of the degree of doctor of philosophy.

The writer wishes to express his thanks to Dr. J. G. Lipman for the suggestion of this problem and for the interest taken in the progress of the work.

Ashley (2) deems it entirely feasible to concentrate the glauconite by electric magnets, while his experiments with a solution of carbon di-oxide, sulfur di-oxide and dilute hydrochloric acid failed to give results of value for the production of commercial potash. A recently reported study at the Maryland Agricultural Experiment Station (9) shows the possibility of composting greensand with inoculated sulfur and manure as a practical means for farmers to make use of the potassium in the greensand.

A number of experiments have been conducted in different European countries with local greensand marls. In America the value of greensand has long been known. In 1819, at least four-fifths of the land in New Jersey around the places where the greensand marl is found, or two-fifths of the whole state was barren according to Morse's American Universal Geography (10). The analyses of the greensand made by Seybert (12) in 1822 shows the existing interest in these early days. A few years later Gordon (6, part 2, p. 5) wrote "It would be difficult to calculate the advantages which the state has gained, and will yet derive from the use of marl. It has already saved some districts from depopulation and increased the inhabitants of others, and may one day contribute to convert the sandy, and pine deserts into regions of agricultural wealth." The prediction of Gordon has come true and much of the land he considered as "deserts" are at present "regions of agricultural wealth." Cook (5) published in 1868 a geological report giving a large number of analyses of greensand samples. In his discussion he ascribes the heavy crops of clover, etc., to the beneficial effects of greensand partly because of the phosphoric acid content and partly because of the sulfuric acid found in it, for "this (sulfuric acid) constituent combines with lime forming plaster" of which the "efficiency as a fertilizer is well known, though the cause of its action is not satisfactorily explained." Although at that time the part played by potassium was not well understood, Cook points out that the peculiarly beneficial effects of greensand on potatoes containing high amounts of potassium, has strengthened the opinion that this constituent of the marl is of high value.

The most interesting greensand deposits of New Jersey and Maryland have been described by a number of writers. Clark (4), Ashley (2, p. 27–58) and others comment similarly on the value of greensand as a fertilizer. Patterson (11) concludes that the marls of Maryland have very little value for commercial extraction of the potassium on account of the great mass of worthless material in these shell marls. Blair (3) while reporting a number of analyses of New Jersey greensand samples concludes that, under the conditions existing in the soil, the potash of the greensand marls becomes gradually available, and since "potash is of especial value to potatoes and to grasses, the benefits derived from marl when used for these crops would lead one to believe that such crops can use the potash of marl to a considerable extent." Lipman and Blair (7) conducted experiments in pots with coarse sand growing barley and buckwheat followed by a crop of soy beans, using greensand as a source of potassium. Barley and buckwheat gave greatly increased yields over the check pots in these experiments. Soy beans fertilized with greensand produced as great a yield of hay as those receiving an application of soluble potassium salts. True and Geise (14) made a study of potassium salts and greensands in sand cultures, using Shive's complete nutrient solution, R_5C_2, as a basis. They conclude that "greensand and greensand marls from Virginia and New Jersey are able to supply sufficient potassium to satisfy the demands of Turkey Red wheat and red clover during the first two months of their growth. This enables them to make a greater dry weight of tops than was seen in similar cultures in which the potassium demand was supplied by potassium chloride, potassium sulfate, and potassium phosphate." Lipman and his co-workers (8) report field experiments with mixtures of greensand marl and inoculated sulfur. The greensand was applied together with acid phosphate and dried blood. The yields obtained with inoculated sulfur alone were as great as the returns from plots receiving a combination of greensand and inoculated sulfur, but the greensand alone compared with the uninoculated sulfur gave slightly greater yields, but the air dry weights produced by a combination of uninoculated sulfur and greensand was lower than the yields produced by either greensand or sulfur alone or a combination of these two constituents.

Ames and Boltz (1) report to have found that the oxidation of sulfur in the soil as well as dried blood liberated potassium. From these studies they conclude that the liberation of potassium was brought about by the salts rather than by the direct action of acidity on the insoluble potassium compounds. McCall and Smith (9) composted greensand with sulfur, soil and manure for the purpose of determining the effect of different composts upon the availability of the potassium of greensand. They inoculated the mixtures with a soil extract known to contain sulfur-oxidizing organisms and concluded, after an incubation period of 23 weeks, that "in composts consisting of greensand, manure, and soil in different proportions, an appreciable amount of potassium of the greensand was made water-soluble." Composts which yielded the largest quantities of water-soluble potassium contained the largest proportions of manure, indicating that nitrogen stimulates sulfur oxidizing bacterial activities.

PLAN OF EXPERIMENT

The experiments reported below were conducted primarily for the purpose of determining the effect of uninoculated sulfur as compared with inoculated sulfur upon the availability of potassium. In the second place the object was to study the possibility of decreasing the large quantities of soil used in earlier experiments, so as to make composting of greensand with sulfur more practical for the farmer or manufacturer, and in the third place to determine the effect of ammonium sulfate upon the rapidity with which the potassium ingreens and becomes available when no soil is used in the mixtures.

The experiments consisted of composting greensand with sulfur and soil in varying proportions and with additions of ammonium sulfate. Commercial sulfur and Penn loam were used. The greensand was from Eatontown, N. J., with a fairly high percentage of potassium and but a trace of calcium. Half of the mixtures were inoculated with infusions known to contain sulfur-oxidizing organisms, and to the other half no sulfur-oxidizing organisms were added. It was found extremely difficult to keep the uninoculated mixtures free from contamination. Although sterilized sulfur was used, much, if not all, of the soil around the New Jersey Experiment Station contains the sulfur-oxidizing organisms. The soil was left unsterilized so as not to change the soil flora, which would have resulted in placing some of the cultures at a too great disadvantage.

The materials were mixed in the following proportions:

COMPOST NUMBER	PARTS OF SOIL	PARTS OF SULFUR	PARTS OF GREENSAND	
1 and 9	100	20	80	
2 and 10	100	40	60	
3 and 11	20	20	160	
4 and 12	20	40	140	
5 and 13	0	20	180	
6 and 14	0	40	160	
7 and 15	0	20	180	0.2 per cent $(NH_4)_2SO_4$ added
8 and 16	0	40	160	0.2 per cent $(NH_4)_2SO_4$ added
9 and 18	0	0	200	

METHODS

All materials were air-dry and mixed thoroughly before the inoculations were made and before the ammonium sulfate was added. A part of each mixture was used for determining the water-holding capacity, Hilgard method. Water equivalent to 60 per cent of the water-holding capacity was then added to the mixtures and they were left standing over night before determinations of hydrogen-ion concentration, relative acidity, sulfates and potassium were made. The cultures were placed in tumblers covered with glassplates and incubated at 28°C.

Water extractions were made of weighed air-dried samples from each compost by shaking 20 gm. of mixture with 200 cc. of distilled water in 1-liter flasks in a shaking machine for 2 hours. The flasks were left standing over night and an aliquot drawn off from each for the pH determinations. The remainder of the contents of the flasks was then filtered until the liquid was clear.

The hydrogen-ion concentrations were at first determined according to the method described by Gillespie and later checked up with a portion of the liquid drawn off after shaking of the mixture with distilled water. The differences, if any, were always very slight. As indicators, the series recommended by Clark and Lubs was used with the apparatus described by Van Alstine (15).

TABLE 1

Composition of greensand and soil used

	MOISTURE	INSOLUBLE RESIDUE	Fe_2O_3 Al_2O_3 MgO Na_2O	K_2O	P_2O_5	CaO	SO_4	pH
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Greensand...........	3.54	54.11	30.79	4.53	1.16	Trace	1.08	6.5
Penn loam...........	2.17	76.04	12.49	0.49	0.17	0.32	0.52	6.7

Determinations of relative acidity were made upon aliquots of the water extract. The liquid was boiled to expel carbon dioxide, cooled, and titrated with 0.1 N NaOH, using phenolphthalein as an indicator.

Potassium determinations were made gravimetrically at the platanic chloride method from aliquots of the water extract. The silicates, iron, aluminum, phosphorus and soluble organic matter were eliminated by evaporation with H_2SO_4, ignition and subsequent precipitation with NH_4OH.

The soluble sulfur was determined by acidifying aliquots of the water extract with concentrated hydrochloric acid and precipitating at the boiling point with barium chloride. The results are calculated as sulfur trioxide (SO_3).

The results recorded are calculated on the moisture-free basis of the soil and greensand. Moisture determinations being made by heating portions of air-dry material and composts for 15 hours at 105° to 108°C.

The greensand and soil were analysed by the official methods at the beginning of the investigation. The results are reported in table 1.

From the hydrogen-ion concentration determinations it may be seen that both soil and greensand were slightly acid. The greensand was rather coarse and therefore crushed in a mortar before mixing with the soil. Mechanical analyses of the greensand and Penn loam used, showing their texture, are given in table 2.

TABLE 2

Mechanical analyses of greensand and soil used

CONSTANTS	GREENSAND	PENN LOAM
	per cent	*per cent*
Fine gravel	0.42	6.75
Coarse sand	18.64	23.92
Fine sand	42.75	26.25
Very fine sand	29.20	26.56
Silt and clay	8.63	16.32

EXPERIMENTAL RESULTS

Acidity and hydrogen-ion concentration

The acidity and hydrogen-ion concentration of the water extracts from each compost were determined at intervals of the inoculated and the uninoculated mixtures. Part of the data obtained are reported in table 3.

It is clear from the data presented that no great differences were found between the cultures with inoculated and uninoculated sulfur. As has been pointed out this was to be expected since it is extremely difficult to keep the inoculated mixtures from contamination under ordinary laboratory conditions. All cultures showed a rapid change in hydrogen-ion concentration and a gradual increase in titratable acidity. After 6 weeks one of the cultures to which soil was added had reached the point of hydrogen-ion concentration at which the potassium of the greensand becomes available, which point was reached by most of the cultures after an incubation period of 9 weeks. From then on the hydrogen-ion concentration changed but slightly, although acidity accumulation went on, showing that sulfur oxidation proceeded. These slight changes in hydrogen-ion concentration indicate that but relatively small amounts of free acid existed and nearly all of the acidity titrated existed in the form of sulfates. The mixtures with soil made somewhat larger quantities of acidity, from the beginning but the intensity of the acid produced was generally less than in the cultures without soil. The quantities of acidity titrated were highest in the mixtures with 50 per cent soil. Addition of ammonium sulfate did not seem to have much influence upon the quantity of acid produced, nor upon the intensity of the acid produced except, possibly, as shown by some slight differences in the uninoculated cultures between those receiving neither soil nor ammonium sulfate and those receiving ammonium sulfate but without soil. There seemed to be sufficient nitrogen available for the needs of the organisms to last until the end of the experiment.

TABLE 3

Accumulation of water-soluble acidity, hydrogen-ion concentration, water-soluble sulfate, and water-soluble in potassium inoculated and uninoculated compost mixtures

NUMBER	COMPOSITION			INITIAL REACTION		REACTION AFTER 18 WEEKS		SO$_3$ PER 10 GM. OF COMPOST				K PER 10 GM. OF COMPOST	
	Soil	Sulfur	Greensand					At start	After 9 weeks	After 15 weeks	After 18 weeks	At start	After 18 weeks
Inoculated													
	parts	*parts*	*parts*	*cc.**	*pH*	*cc.**	*pH*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1	100	20	80	0.05	6.6	37.6	2.3	1.32	200.24	218.99	233.08	1.24	7.91
2	100	40	60	0.05	6.6	42.0	2.2	1.30	186.74	211.25	228.66	1.08	7.39
3	20	20	160	0.05	6.6	26.8	2.2	1.48	158.22	184.96	200.85	0.72	11.79
4	20	40	140	0.05	6.6	31.2	2.0	1.48	184.99	204.89	206.92	0.56	12.68
5	0	20	180	0.06	6.6	29.6	2.0	1.70	161.16	207.47	212.07	0.62	16.48
6	0	40	160	0.08	6.6	25.8	2.0	1.68	114.58	164.81	184.47	0.53	11.47
7	0†	20	180	0.10	6.6	24.0	2.1	3.72	147.50	184.60	198.89	0.36	12.71
8	0†	40	160	0.08	6.6	30.0	2.0	3.40	150.18	193.92	215.27	0.20	11.71
17	0	0	200	0.10	6.5	0.1	6.3	1.08	6.60	7.90	8.02	0.25	0.29
Uninoculated													
9	100	20	80	0.05	6.6	31.2	2.2	1.32	101.66	145.44	197.86	0.54	7.23
10	100	40	60	0.05	6.6	38.2	2.4	1.30	163.74	192.35	218.15	0.82	6.07
11	20	20	160	0.05	6.6	24.2	2.1	1.48	146.92	161.99	179.14	0.27	11.07
12	20	40	140	0.05	6.6	31.4	2.1	1.48	173.01	184.43	199.92	0.45	11.31
13	0	20	180	0.06	6.6	19.3	2.3	1.70	101.02	141.54	170.77	0.15	12.27
14	0	40	160	0.08	6.6	17.2	2.3	1.68	113.85	159.17	149.55	0.27	11.31
15	0†	20	180	0.09	6.6	27.0	2.2	3.72	142.19	158.52	192.40	0.18	13.80
16	0†	40	160	0.08	6.6	22.8	2.3	3.40	130.95	182.62	190.86	0.18	12.18
18	0	0	200	0.10	6.5	0.1	6.2	1.08	6.58	7.22	8.83	0.25	0.36

* Acidity expressed in cc. 0.10 norm. NaOH required to neutralize acidity of water extract of 10 grams compost.

† 0.2 per cent ammonium sulfate added.

SULFATE FORMATION

The production of water-soluble sulfates during the first weeks of incubation was very rapid in all mixtures. From the results recorded in table 3 it may be seen that after an incubation period of 15 weeks the inoculated mixtures had produced more sulfates than the uninoculted composts.

This gain, however, was largely lost after 18 weeks, due to the fact that the sulfate accumulation was slow between the 9th and 18th weeks of incubation. The reason for the slower accumulation may possibly be that the sulfates and free acid formed at first do not react with the mineral constituents present, or do so only very slightly, but when sufficient amounts are produced a comparatively rapid reaction takes place with the extremely small particles, while later on the slower reaction takes place with the more coarse material. In the course of this process a part of the sulfates unite with bases of the silicates and become insoluble in water. The more rapid attack of small particles would indicate that finely ground greensand yields greater amounts of the water soluble potassium. An experiment conducted with material of different fineness showed distinctly that the finer material used, the faster the reaction takes place. No attempt was made to study the possible interchange of bases in this relatively slow reaction process.

WATER-SOLUBLE POTASSIUM

Water-soluble potassium determinations were made at the beginning of the experiment, after 1, 3, 6, 9, 15 and 18 weeks. The data secured after 18 weeks are recorded in table 3.

The increase in water-soluble potassium was gradual in all cultures. The amounts during the first 12 weeks were small.

Until the fifteenth week the inoculated cultures were ahead of the uninoculated, but after 18 weeks not much difference was apparent. The possible reason has been pointed out by the discussion of the accumulation of water soluble sulfates.

The total water-soluble potassium seemed to be greatest in all mixtures without soil additions, but if calculated on the basis of the per cent of total potassium present, this is reversed.

It is necessary that a certain degree of acidity be produced before the potassium becomes water-soluble. The amounts of sulfates formed do not necessarily have to reach a certain quantity, but the acidity produced has to be of a certain intensity. The point at which the potassium of the greensand becomes more rapidly water soluble lies between the pH values 2.7 and 2.3, as is shown by determination made from greensand extracts treated with dilute sulfuric acid. Nevertheless, the quantities of sulfates formed have a close relation to the intensity of the free acid formed.

The curves in figure 1, show clearly the relation between hydrogen-ion concentration, acidity accumulation, sulfate formation and water-soluble potassium in two of the inoculated mixtures.

It seems evident that a part of the acid or acid sulfates formed in culture 1 reacted with the soil constituents, while in culture 3 these sulfates or the free acid acted more directly upon the potassium of the greensand. It would seem that most rapid and thus most economical results would be obtained if a part of the composts were used for inoculaton of new compost mixtures, eliminating thereby the long period of incubation before a certain hydrogen-ion concentration and a certain acidity accumulation is reached.

As has been pointed out above, the total water-soluble potassium seemed to be greatest in all mixtures containing no soil, but from the data presented in table 4 it is evident that a smaller percentage of the total potassium present had been liberated in the cultures without additions of soil. It should be kept in mind that the greensand contained but 4.63 per cent of total potassium and much of the materials present react with the acid or acid compounds formed. Besides, the reaction was not complete and still continuing at the end of 18 weeks. Since only a small amount of the sulfur was oxidized it does not seem necessary to add such large quantities of sulfur as was done in this experiment. The cultures to which 40 gm. of sulfur were added had accumulated almost

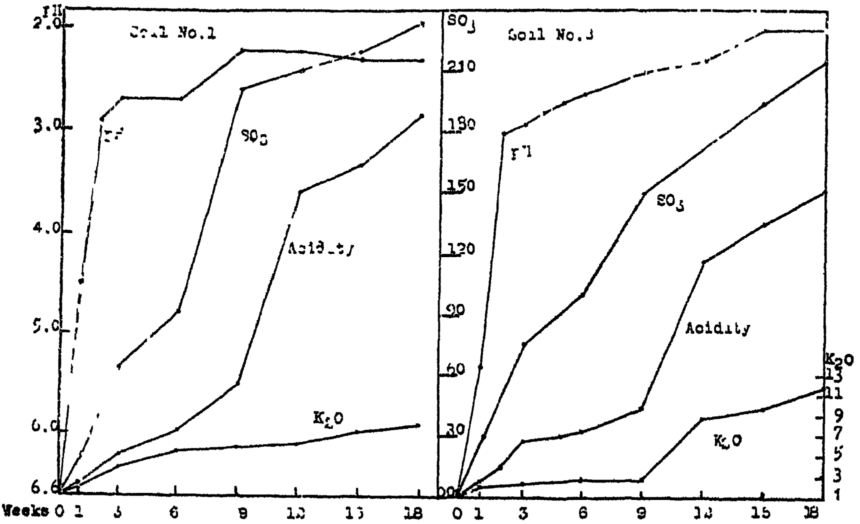


FIG. 1. GRAPHS SHOWING THE RELATION BETWEEN HYDROGEN-ION CONCENTRATION SULFATES, ACIDITY AND WATER SOLUBLE POTASSIUM IN COMPOST MIXTURES NUMBERS 1 AND 8

TABLE 4

Total sulfur oxidized and total water-soluble potassium obtained

CULTURE NUMBER	SULFUR IN MIXTURE	SULFUR OXIDIZED		POTASSIUM K_2O IN MIXTURE	SOLUBLE POTASSIUM	
	gm.	*gm.*	*per cent*	*gm.*	*gm.*	*per cent*
1	20	1.864	9.32	3.624	0.1582	4.37
2	40	1.830	4.57	2.718	0.1478	5.44
3	20	1.606	8.03	7.248	0.2358	3.26
4	40	1.658	4.14	6.342	0.2536	3.99
5	20	1.698	8.49	8.154	0.3296	4.04
6	40	1.474	3.68	7.248	0.2294	3.17
7	20	1.592	7.96	8.154	0.2542	3.12
8	40	1.723	4.31	7.248	0.2342	3.23
9	20	1.578	7.89	3.624	0.1446	3.92
10	40	1.746	4.36	2.718	0.1214	4.47
11	20	1.442	7.21	7.248	0.2214	3.07
12	40	1.588	3.97	6.342	0.2262	3.57
13	20	1.369	6.85	8.154	0.2454	3.01
14	40	1.196	2.99	7.248	0.2262	3.13
15	20	1.540	7.70	8.154	0.2760	3.38
16	40	1.528	3.82	7.248	0.2435	3.36
17	None			9.06	0.0007	0.078
18	None			9.06	0.0006	0.067

exactly the same amount of sulfates as the culture to which 20 gm. of sulfur were added. The relation between the percent of potassium liberated and the per cent of sulfur oxidized was roughly 1:2. It seems therefore, that approximately 10 per cent of sulfur of the total greensand is sufficient to secure satisfactory yields.

SAND CULTURES

The per cent of water-soluble potassium was low in all cases, but it seemed sufficient to supply plants with the necessary amounts of potassium. In order to test whether or not the available potassium would be sufficient to support plants with this necessary plant food element, pot cultures were conducted with soy bean plants. Since, as has been shown by earlier investigations, potassium absorption is greatest during the first part of the growing period of the plants, soy beans were grown for only 6 weeks. Soy bean plants ordinarily bloom in the greenhouse between the fourth and fifth week and the growing period of 6 weeks would be enough to test the value of the material for these plants.

Although it is known that the ordinary glazed earthenware pots furnish very small amounts of soluble impurities among which are potassium compounds, it was thought that the differences among the cultures would be large enough to give this test practical value. As a source of the necessary nutrient salts Shive's cultural solution R_5C_2 was used.

Each pot contained 5 kgm. washed quartz sand. Treatments, in triplicate series were as follows:

1. None
2. Dry greensand.
3. Shives cultural solution R_5C_2.
4. Composted greensand.
5. Shive's solution, except potassium, plus dry greensand.
6. Shive's solution, except potassium, plus composted greensand.

The greensand substituted for the potassium in Shive's cultural solution was calculated to be approximately that necessary to supply an amount of potassium equivalent to that in the solution. The inoculated greensand had been composted for a period of 18 weeks previous to the application. The results from the plants grown in these cultures would thus be comparable with the sults of the plants grown in cultures with the easily available potassium of Shive's cultural solution.

The soy bean seeds were selected for size and germinated in sand. The seedlings were then selected again to be as nearly alike as possible and planted when about two inches high. After the planting the cultures were inoculated with a few cc. of a water extract known to contain nodule forming bacteria. The yields obtained after a growing period of 6 weeks are presented in table 5 in the form of dry weights of tops, height of plants, number of pods produced, and the pH values at the beginning and at the end of the experiment.

It will be noted that the dry greensand alone had some influence on the growth of these soy bean plants.

The dry greensand substituted for the potassium in the cultural solution was apparently not able to replace the potassium fully for the needs of these plants in this period of growth, but the composted greensand seemed to render sufficient potassium available for their needs. In fact, the cultures receiving greensand as a substitute matured earlier and seemed more vigorous than the plants in Shive's nutrient solution.

From the numbers of pods produced, it can be seen that the plants receiving greensand as a substitute for the potassium in Shive's solution would yield also more seeds, showing that their earlier maturity was profitable instead of detrimental, although slightly less hay was produced as compared with the plants grown in the R_5C_2 solution. In addition, more and larger nodules were noticed on the roots of all plants receiving potassium in the form of greensand.

TABLE 5

Yields of tops of soy beans grown in sand cultures, with Shive's nutrient solution as a basis, with the potassium of the solution replaced by the potassium of composted greensand

CULTURE NUMBER	TREATMENT	DRY WEIGHTS OF TOPS	AVERAGE HEIGHT	NUMBER OF PODS	REACTION		NODULES
					Initial	Final	
		*gm.**	*cm.*		*pH*	*pH*	
1	None	0.856	8	Bloom	5.7	6.8	Small
2	Greensand	1.132	9	2	5.3	6.1	Large
3	R_5C_2	3.356	14	4	5.4	6.4	Few
4	Composted greensand	1.206	8.5	2	5.4	5.8	Abundant
5	R_5C_2, greensand	2.501	13	6	5.0	6.5	Few, but large
6	R_5C_2, composted greensand	3.299	14	9	4.8	6.4	Few, but large

* All results given are averages of 3 cultures.

CONCLUSIONS

1. In composts consisting of greensand and sulfur, small amounts of potassium are liberated.

2. No great differences occured between inoculated and uninoculated sulfur-greensand mixtures, because there was contamination of the uninoculated mixtures.

3. The potassium of the greensand is made available at a certain hydrogen-ion concentration, lying between the pH values 2.7 and 2.3.

4. There is a definite relation between the hydrogen-ion concentration and sulfate formation, and the water-soluble potassium.

5. Soy bean plants grown in quartz sand and with Shive's nutrient solution in which composted greensand was substituted for the potassium of the cultural solution are able to make as great a dry weight of tops as in similar cultures in which the potassium was supplied in the form of potassium phosphate.

Soy bean plants receiving composted greensand as a source of potassium matured earlier and yielded more seeds than the plants grown in Shive's cultural solution.

REFERENCES

(1) Ames, J. W., and Boltz, G. E. 1919 Effect of sulfofication and nitrification on potassium and other soil constituents. *In* Soil Sci., v. 7, p. 183–195.

(2) Ashley, G. H. 1917 Notes on the greensand deposits of the Eastern United States. U. S. Geol. Sur. Bul. 660 B.

(3) Blair, A. W. 1916 The agricultural value of greensand marl. *In* N. J. Agr. Exp. Sta. Cir. 61.

(4) Clark, B. W. 1893 A preliminary report on the Cretaceous and Tertiary formations of New Jersey. *In* N. J. Geol. Sur. Ann. Rpt. 1892, p. 169–239.

(5) Cook, G. H. 1868 Geology of New Jersey. State Geological Survey, New Brunswick, N. J.

(6) Gordon, Th. 1830 History and Gazeteer of New Jersey. (p. 442–443 in 15.)

(7) Lipman, J. G., and Blair, A. W. 1917 Vegetation experiments on the availability of phosphorus and potassium compounds. *In* N. J. Agr. Exp. Sta. Ann. Rpt. 38, p. 353–368.

(8) Lipman, J. G., Blair, A. W., Martin, W. H., and Beckwith, C. S. 1921 Inoculated sulfur as a plant-food solvent. *In* Soil Sci., v. 11, p. 87–92.

(9) McCall, A. G., and Smith, A. M. 1920 Effect of manure-sulfur compost upon the availability of the potassium of greensand. *In* Jour. Agr. Res., v. 19, p. 239–256.

(10) Morse, Jedidiah 1819 American Universal Geography, 7th ed. Lincoln and Edmands, Boston and G. Clark, Charleston, Mass.

(11) Patterson, H. J. 1906 Results of experiments on the liming of soils. Md. Agr. Exp. Sta. Bul. 110.

(12) Seybert, H. 1826 Analyses of the green earth from Rancocas creek, New Jersey. *In* Mem. Phila. Soc. Prom. Agr., v. 5, p. 18–46.

(13) Shreve, R. N. 1920 Potash recovery in New Jersey. *In* Chem. Age, v. 4, p. 69–73.

(14) True, R., and Geise, F. W. 1918 Experiments on the value of greensand as a source of potassium for plant culture. *In* Jour. Agr. Res., v. 15, p. 483–492.

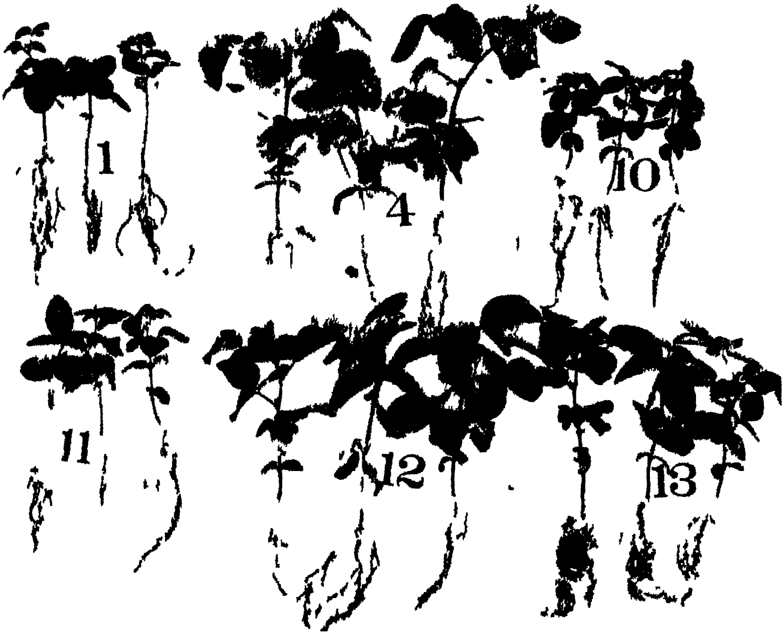
(15) Van Alstine, E. 1920 The determination of hydrogen-ion concentration by the colorimetric method and an apparatus for rapid and accurate work. *In* Soil Sci., v. 10, p. 467–479.

PLATE 1

SOY BEAN PLANTS GROWN IN QUARTZ SAND TREATED AS FOLLOWS

1. None.
4. R_5C_2.
10. Composted greensand.
11. Greensand.
12. R_5C_2 and composted greensand.
13. R_5C_2 and greensand.

PLATE 1



MICROBIOLOGICAL ANALYSIS OF SOIL AS AN INDEX OF SOIL FERTILITY: III. INFLUENCE OF FERTILIZATION UPON NUMBERS OF MICROÖRGANISMS IN THE SOIL.[1]

SELMAN A. WAKSMAN[2]

New Jersey Agricultural Experiment Stations

Received for publication December 6, 1921

HISTORICAL

The majority of the earlier investigators on the numbers of bacteria in the soil including Remy, Hiltner and Störmer, Löhnis, Kruger and Heinze, Brown, Conn, etc., have concerned themselves very little with making a direct practical application of their investigations to actual soil conditions. These investigations led merely to establishing the fact that numbers of microörganisms in the soil are more or less uniform [although even this is questionable, as pointed out in the first paper of this series (16)], that the application of stable manure and green manure increase the number of microörganisms in the soil, that continued drying or treatment with volatile antiseptics has a depressing effect, and subsequent moistening of the soil has a stimulating effect. Engberding (2) found the number of bacteria in the soil to run parallel with the moisture-content of the soil and Fabricius and Feilitzen (3) found a parallelism with soil temperature.

Rahn (14) found that the crop and the system of cropping exert a direct influence upon the numbers of microörganisms but Engberding (2) has shown that, whenever a difference was observed due to cropping, it could be accounted for by the moisture content of the soil. Hiltner and Störmer (7) found that fallowing resulted in a decrease in numbers, but Krüger and Heinze (8) obtained an increase. The addition of organic matter, in the form of dextrose, straw and green manure results, according to Engberding (2), in a large increase in numbers, followed by a decrease, so that in a few months the level of the control is reached. Ammonium sulfate, sodium nitrate, potassium sulfate, lime and magnesium were found by Engberding to have a slight stimulating effect upon numbers of microörganisms in the soil, while superphosphate was without influence.

A detail review of the earlier investigations on the subject is found in the Handbuch of Löhnis (11).

Only in very few instances has there been an actual attempt made to correlate numbers of microörganisms in the soil with crop production. In most cases the results have been rather negative. This has been due to the fact that usually few, unrepresentative soil samples have been used, and to the fact that the methods have not been standardized, so that the results obtained, even when positive, were so variable as to be of questionable importance. The desirability of a correlation between the information obtained from the study of bacterial activities and crop production has been pointed out by Fischer (4).

---

[1] Paper No. 101 of the Journal Series, New Jersey Agricultural Experiment Stations, Department of Soil Chemistry and Bacteriology. This paper will appear in RUTGERS COLLEGE STUDIES, vol. 1.

[2] The author wishes to thank Mr. J. S. Joffe and Mr. R. L. Starkey for assistance in the taking of the samples and in pouring the plates, and Dr. J. G. Lipman and Prof. A. W. Blair for permission of using the plots and data for the productivity and history of plots.

In a few cases, however, definite correlations have been obtained between the number of microörganisms and soil productivity, but, unfortunately, these observations have been rather sporadic and not followed up in a systematic way. Neller (12), for example, obtained a noticeable correlation between crop yield, oxidizing power of the soil, nitrate production and bacterial numbers, but not between crop yield and ammonia accumulation, thus foreshadowing the reliability of the three first bacteriological functions and the unreliability of the ammonia-producing power of a soil as an index of its fertility. A definite correlation between bacterial numbers and crop yield has also been recorded by Noyes and Conner (13) and others.

EXPERIMENTAL

In the two previous papers in this series, the important principles to be observed in the study of numbers of microörganisms in the soil have been discussed. This paper deals with the influence of soil fertilization upon the numbers of microörganisms.

Before making an extensive study of the numbers of microörganisms in the soil, some information had to be obtained on the condition of equilibrium in the soil and on the variability of microörganisms in various soil samples kept under the same conditions. For that purpose a greenhouse soil was air-dried, 600-gm. portions were placed in each of six small, glazed earthenware pots, and enough water added to bring it to 70 per cent of the moisture-holding capacity of the soil (28 per cent). The soil was stirred every 7–10 days, with the addition, at those intervals, of the proper amount of water to keep the soil at constant moisture. Three of the pots were left untreated, and, to three pots 22.5 mgm. of sulfur and 90 mgm. of rock phosphate were added. The numbers of microörganisms exclusive of fungi, were determined at various intervals and the results are given as averages in table 1 and figure 1.

When the soil is air-dried, there is a sudden drop in the number of microorganisms to one-half the number of the fresh soil, which we would expect due to the sudden elimination of many of the non spore-forming bacteria. When the soil is again moistened, there is a rapid increase in bacterial numbers, reaching not only the original value, but increasing to more than double. This is due to the influence of drying upon the soil, which served as a stimulus of the bacterial activities in the soil. The increase in numbers is somewhat uneven in the different pots (see table). The rise in numbers continues till the second sampling which took place two weeks later, and is then followed by a fall in numbers.

We thus find a rapid rise in numbers, due to the effect of drying which lasts for a short period of time and followed by a drop. The drop is not so sudden as the rise, indicating that the numbers come again only slowly to a new equilibrium. Since the stimulus was not due to the introduction of new food material, but merely to a change in the soil condition, we would expect that the new equilibrium once established will be at a lower plane than the first one. The stimulated activity of microörganisms will result in a decomposition of the soil organic matter which will leave the soil poorer than it was before and thus

TABLE 1

*The course of change of numbers of microörganisms in the soil**

DATE	SOIL CONDITION	COLONIES (IN THOUSANDS) PER GRAM OF SOIL						
		1a	1b	1c	2a	2b	2c	Average
1/ 7/21	Fresh soil	21,750†						21,750
1/21/21	Air-dried soil	10,700†						10,700
1/25/21	Soil was moistened on 1/21/21	44,260	35,320	50,590	49,680	34,330	47,880	43,677
2/ 8/21	Moist	49,210	37,210	49,320	46,610	38,660	37,580	43,098
2/25/21	Moist	33,240	37,220	38,750	25,840	29,630	25,840	31,753
3/18/21	Moist	23,680	21,330	16,700	26,534	26,070	24,470	23,131
4/13/21	Moist	21,660	19,130	15,060	26,000	23,000	22,200	21,175
5/13/21	Moist	16,000		9,200				12,600
7/ 8/21	Moist	13,000	11,360	5,400	8,200	8,800	8,600	9,227
12/12/21	Moist	5,600	4,800	4,600	4,700	5,200	5,600	5,083

* 1a, 1b, 1c are the pots containing only soil; 2a, 2b, 2c contain, in addition to 600 gm. of soil, 22.5 mgm. sulfur and 90 mgm. rock phosphate. Numbers represent all the colonies, except fungi, in thousands per gram, on the basis of soil dried to constant weight.

† These figures represent the averages of the uniform soil previous to distribution in the pots.

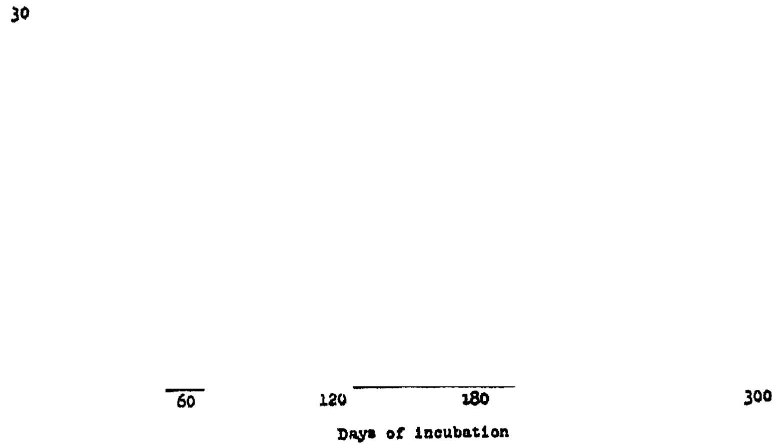


FIG. 1. COURSE OF NUMBERS OF MICROÖRGANISMS IN SOIL PLACED IN CONTAINER AND KEPT IN THE LABORATORY AT UNIFORM MOISTURE CONTENT

able to harbor fewer microörganisms. With each repeated stimulus (shift of the unstable equilibrium), if no other nutrients in the form of nitrogenous fertilizers or organic matter are added, there will result a new equilibrium at a lower stage than the previous one. This will parallel the phenomena of unproductivity of a constantly fertilized soil.

It is interesting to note that on July 8, only six months after the soil had been taken from the greenhouse, air-dired, and again moistened, the numbers dropped to a level as low or lower than that in the original air-dried soil. It is also of interest to note that pot 1c, in which the bacterial numbers reached the highest peak, dropped down to the lowest point in numbers, while the pots 2a, 2b and 2c, which had an addition of phosphate, fell down to a lower level than pots 1a and 1b. This would suggest a solution of the problem, based not upon toxin production, activities of protozoa, or any other theory adapted at one time or another to explain this common soil phenomenon, but a mere principle based upon physico-chemical laws, namely that of mass action and equilibrium. This subject is of so great importance that merely to touch upon it is to overlook its part in soil microbiological activities. It will be the subject of one of the following papers in this series. A detailed study of the influence of air drying upon the bacteriological activites in the soil is found in the papers of Rahn (14) and Heinze (6).

For the study of the influence of fertilization upon the numbers of microorganisms in the soil, a series of plots, used by the Soil Department of the New Jersey Agricultural Experiment Stations for the study of availability of nitrogenous fertilizers were selected. These plots are one-twentieth of an acre in size and were laid out in 1908; since then they have been under the same system of fertilization, one-half of the plots receiving various fertilizer and manure treatments without lime, the other receiving the same treatment and lime. The soil is a Sassafras loam with a small amount of coarse gravel. The crop rotation used consists of corn, oats, wheat, and timothy, with some supplementary residual crops. When these studies were made, the plots were under timothy.

These plot experiments, as well as the results of chemical analysis of the soil, are described in detail in the papers of Lipman and Blair (9, 10) in which the crop yields obtained for the first ten years, are reported. Only those data will be reported here which have a direct bearing upon the problem at hand. Of the 20 unlimed and 20 limed plots, ten were selected, namely 4A, 5A, 7A, 9A, 11A, 18A, 19A, 7B, 11B, 19B. The following tables give a description of fertilizer treatment, composition and crop yields for the plots selected.

It will be noted that the highest crop yields were given by Plot 18A receiving manure, sodium nitrate and minerals, and 5A, the plot that received manure alone, in addition to minerals. Next to the two manured plots came the two plots receiving minerals, ammonium sulfate and lime (11B) and sodium nitrate (9A).

TABLE 2

Fertilizer treatment and carbon and nitrogen content of plots

PLOT NUMBER	FERTILIZER TREATMENT ON ACRE BASIS	TOTAL CARBON*		TOTAL NITROGEN	
		1913	1917	1913	1917
		per cent	*per cent*	*per cent*	*per cent*
4A	Minerals only (640 lbs. acid phosphate and 320 lbs. potassium chloride)	1.36	1.26	0.1180	0.1088
5A	Minerals and 16 tons of cow manure	1.38	1.44	0.1002	0.1185
7A	Nothing	0.95	0.93	0.0790	0.0785
9A	Minerals and 320 lbs. $NaNO_3$	1.15	1.13	0.0921	0.0975
11A	Minerals and $(NH_4)_2SO_4$ equivalent to 320 lbs. $NaNO_3$	1.14	1.21	0.0977	0.0904
18A	Minerals and 16 tons of cow manure and 320 lbs. $NaNO_3$	1.47	1.36	0.1088	0.1170
19A	Minerals only (same as 4A)	1.29	1.01	0.0980	0.0872
7B	Same as 7A, with lime†	1.17	1.02	0.0879	0.0821
11B	Same as 11A, with lime†	1.02	1.06	0.0801	0.0819
19B	Same as 19A, with lime†	1.11	0.95	0.0809	0.0784

* The average of analysis of 5 plots in 1908 when the plots were laid out gave 1.22 per cent of carbon and 0.1118 per cent of nitrogen.

† The fertilizer treatment of the B plots is the same as of the corresponding A plots, with the addition of 1 ton of ground limestone per acre in 1908, 2 tons in 1913 and 2 tons in 1918.

TABLE 3

*Yield of dry matter for the years 1908–1921**

PLOT NUMBER	TOTAL FOR 5 YEARS 1908–1912	TOTAL FOR 5 YEARS 1913–1917	TOTAL FOR YEARS 1918 AND 1920†	YIELD FOR 1921‡	TOTAL FOR 13 YEARS
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*
4A	13,874	8,800	5,964	1,270	29,908
5A	26,119	21,130	9,322	3,970	60,541
7A	7,352	3,950	3,842	151	15,295
9A	21,879	17,745	7,984	2,880	50,488
11A	21,595	12,805	2,161	2,170§	38,731
18A	29,362	23,140	10,584	4,340	67,426
19A	13,305	7,610	5,442	870	27,227
7B	11,645	9,145	5,799	650	27,239
11B	22,018	19,110	9,078	3,620	53,826
19B	12,098	10,440	7,264	1,650	31,452

* Total of dry matter (hay, straw, grain) is given as pounds per acre.

† Heavy rainfalls delayed the harvesting of the crop of 1919 causing heavy loss of the grain; the results for that year are, therefore, rather misleading, and are for that reason left out entirely.

‡ Pounds of hay per acre.

§ The second crop, on this plot, amounting to 1720 pounds per acre, consisted entirely of crab grass; the actual yield of timothy, on this plot, was only 450 pounds per acre.

TABLE 4

*Numbers of microörganisms in the soil on May 20, 1921**

4A			5A			7A			9A			11A			18A			19A			7B			11B			19B		
B+A	A	F	B+A	A	F	B+A	A	F	B+A	A	F	B+A	A	F	B+A	A	F	B+A	A	F	B+A	A	F	B+A	A	F	B+A	A	F
49*	2	4	196	41	2	36	13	1	98	21	0	19	2	6	136	33	3	56	17	1	79	24	3	82	21	1	119	21	2
78	9	3	198	28	6	46	18	3	74	17	4	35	3	7	143	38	3	51	12	1	96	23	0	78	17	1	112	29	3
109	12	3	181	32	2	42	12	1	108	26	0	25	3	5	152	42	2	64	12	3	71	19	3	107	33	1	111	33	0
69	8	5	145	36	7	33	10	3	106	20	2	34	4	4	122	31	3	56	10	3	79	18	2	108	22	2	120	28	2
46	6	3	152	23	3	45	16	1	92	23	2	27	6	4	131	28	1	36	9	3	95	28	1	96	26	1	134	32	0
78	4	2	168	35	5	37	10	4	121	28	1	24	6	5	143	36	3	71	16	3	83	23	0	103	24	0	136	31	3
53	6	3	169	26	4	53	17	3	97	22	1	23	4	3	146	27	2	78	12	0	82	29	0	83	28	2	121	28	1
84	15	1	178	24	2	46	9	1	108	19	1	40	8	1				50	16	2	93	24	2	111	27	0	122	24	0
71	7	3	247	32	3	41	12	2	68	14	2	18	6	3				59	14	2	96	23	3	92	18	1	114	25	2
54	4	3	224	39	3	45	12	2	92	32	1	39	9	1							98	24	2	102	30	3	124	34	2
69	14	2	153	23	3	61	16	1	115	23	4	32	3	5	156	31	2	54	8	2	95	29	1	106	24	1	125	27	1
81	19	2	146	19	5	45	12	2	104	19	2	47	3	5	168	41	3	102	13	2	86	32	2	90	22	1	98	28	2
74	8	3	144	24	3	58	14	2	78	13	2	32	2	3	156	32	3	56	11	3	104	36	5	104	26	1	85	26	2
65	11	0	143	28	5	53	15	1	112	24	2	38	2	5	166	36	2	61	17	3	85	26	0	112	29	2	81	24	2
64	10	2	148	23	4	46	13	3	129	32	3	24	1	7	178	42	3	98	27	2	64	21	2	132	31	0	98	37	2
75	9	3	131	21	3	51	8	6	133	23	0	33	8	9	148	30	2	46	8	3	88	28	1	116	29	1	106	32	2
77	8	3	143	24	3	61	16	1	105	28	2	21	4	4	196	32	3	85	22	1	78	24	3	88	18	2	116	34	0
66	9	0	122	18	6	46	12	2	97	24	4	17	2	5	163	26	2	115	19	3	84	24	1	91	24	1	103	35	2
75	17	4	128	18	3				128	18	2	29	2	7				68	16	2	91	26	0	132	24	2	129	33	2
54	7	4	107	17	4							14	4	3										158	32	3	102	32	1
104	18	4	84	17	3	48	14	3	98	16	1	22	0	4	94	22	2	65	16	1	72	18	2	107	26	1	116	29	1
58	6	2	102	23	2	59	18	1	132	22	3	36	3	5	122	35	2	62	22	2	76	25	2	92	24	2	109	29	2
51	4	2	58	6	4	59	11	6	111	13	4	12	2	8	116	37	2	51	12	4	93	37	3	91	17	2	118	28	1
86	6	1	62	9	2	51	15	2	139	15	1	24	2	3	128	39	4	62	16	2	78	19	0	92	16	2	126	30	2

*The numbers in tables 4–12 indicate the total number of colonies developing on the plate. The actual number will be obtained by multiplying by the proper dilution (100,000 for B and A and 1000 for F).

53	8	1	47	7	5	39	6	1	146	14	1	22	3	6	138	49	2	47	11	6	66	12	2	118	20	1	148	42	0
86	12	1	124	23	3	39	10	4	133	12	2	10	2	3	126	32	2	61	15	4	86	24	2	120	32	0	151	41	2
73	7	2	77	20	3	46	9	2	105	5	1	13	3	6	136	31	3	72	21	1	79	22	3	121	37	2	142	32	1
75	11	3	105	21	4	52	12	0	91	7	1	19	2	7	142	41	2				81	18	2	86	19	2	180	41	1
																					82	23	0	86	17	2	122	31	2
																								94	16	1	119	38	3
66	8	2	93	29	4	41	15	2	67	4	1	18	3	7	191	32	2	41	11	1	69	13	2	122	26	3	122	32	2
89	7	3	128	38	5	48	16	3	85	9	0	28	3	4	152	31	1	36	13	1	53	16	2	156	31	2	132	32	2
76	4	3	72	19	3	52	20	2	82	7	1	45	5	3	163	37	1	73	16	1	86	19	1	112	29	2	136	33	1
68	10	4	87	22	3	38	20	2	93	9	1	42	6	8	136	26	2	53	13	1	68	21	2	116	23	5	128	34	2
74	8	2	102	31	5	46	15	0	99	13	2	41	3	2	178	38	2	64	13	3	88	23	2	113	22	2	105	28	2
102	12	5	98	28	3	39	13	2	103	18	1	31	4	5	164	28	5	69	15	2	73	21	0	149	26	1	102	29	1
117	11	2	142	36	6	37	16	3	71	7	2	28	2	4	157	38	2	52	10	4	78	22	0	96	19	2	136	30	1
88	14	5	68	26	3	51	17	6	64	6	1	32	2	6	182	27	2				71	23	2	107	33	2	142	28	0
																					69	15	2				132	32	2
																					58	16	2				113	22	2
105	13	2	112	26	4	54	16	3	109	22	1	33	4	3				69	19	3	46	13	1	68	19	4	182	46	3
83	9	2	116	24	2	47	10	1	71	18	3	32	6	3				56	14	2	68	23	0	79	26	3	108	32	0
78	12	3	84	17	3	31	13	5	102	25	0	18	2	4				78	16	4	66	26	0	69	22	1	109	26	3
53	6	3	83	18	4	62	18	6	77	20	1	42	11	4				70	14	2	88	23	0	90	23	1	152	29	1
98	8	3	97	26	3	58	20	2	76	15	2	32	8	2				67	18	5	73	31	1	61	14	3	148	29	3
92	8	4	88	21	4	43	9	2	88	17	0	23	7	8				44	9	3	68	17	0	85	28	4	165	27	3
62	7	3	108	18	5	45	13	1	81	24	5	12	2	0				38	11	4	69	18	3	76	19	4	132	35	3
68	9	1	118	22	5	36	8	1	106	22	3	37	2	4				89	19	2				65	18	3			
			123	26	2																								
			127	21	2																								

The poorest yields were obtained from the unfertilized plot (7A) followed by those that received either lime alone (7B) or minerals alone (4A and 19A). The plot receiving ammonium sulfate alone gave rather good yields the first 5 years, but gradually dropped down due to increasing acidity from the yearly application of the fertilizer, as seen from table 13 where the reactions are given.

The plots were sampled 4 times throughout the summer, May 20, June 27, July 18, and September 22. The first set of samples was taken when the soil had not yet had a chance to dry out, the second set of samples was taken after a prolonged dry spell, and the third and fourth soon after brief heavy rainfalls, at the end of dry periods. Fifteen to twenty individual samples were taken from different parts of each plot by means of a 1-inch sampling tube from the upper 6 inches of soil and mixed into 5 composite samples which were used for the determinations, except on June 27 when only 4 samples were prepared for each plot.

The methods of sampling, plating, etc., were given in the previous paper (17). The moisture content and soil reaction (5, 1) at the various dates of sampling are found in the summary table 13. By mistake, the soil moisture was determined only roughly in the first set of samples.

Tables 4, 5, 6, 7, 8, 9, 10, 11 and 12 contain the numbers of microörganisms obtained, by the plate method, on the different dates of sampling. The first four tables (4, 5, 6, 7) contain all the microörganisms developing on the albumen agar plate; the three columns for each plot have the following designations: B + A = bacteria and actinomycetes, indicating all the organisms developing on the plate, except the fungi; A = actinomycetes; F = fungi. Tables 8, 9, 10, 11 contain the numbers of microörganisms, except the fungi found on July 18, 1921 and September 22; the fungi for the samples taken on the corresponding dates were determined by the use of the special acid medium (17) having a pH = 4.0 with a dilution of 1:1000, and are briefly summarized in table 12.

The dilutions used in counting the total number of microörganisms were in all cases 100,000. The figures designate the number of colonies on the plate. The plates were always incubated 3 days for the special fungus media and 7 days for the bacteria and actinomycetes at 25–27°C. The total number of microörganisms per gram of moist soil can then be obtained by merely multiplying the number of colonies by 100,000. By making another multiplication by a factor allowing for the moisture content of the sample, as given in table 13, the numbers are obtained on the basis of air-dried soil. 10 plates (8–9 plates for table 9) were poured for each soil sample using synthetic agar (modified albumen). At first all the colonies, except the fungi, were counted, with the naked eye and reported under column B + A. The actinomycetes were then counted and reported under A, while the fungus colonies are given under F. Where plates were badly overgrown with fungi, particularly in the case of certain *Mucorales* which prevent any extensive development of bacteria and actinomycetes, the plates were discarded and not included in the count. Each

TABLE 5

Summary of results reported in table 4

PLOT NUMBER	MEAN			σ			C.V.			Em		
	B+A	A	F	B+A	A	F	B+A	A	F	B+A	A	F
	colonies	*colonies*	*colonies*				*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
4A	74.9 ± 1.7	9.2 ± 0.4	2.6 ± 0.12	17.0 ± 1.2	3.7 ± 0.4	1.2 ± .09	22.7 ± 1.6	40.6 ± 4.6	46.1 ± 3.5	2.3	4.1	4.6
5A	124.5 ± 4.6	23.2 ± 0.76	3.7 ± 0.12	46.8 ± 1.5	7.7 ± 0.6	1.25 ± .09	37.6 ± 1.2	33.2 ± 2.6	33.8 ± 2.4	3.7	3.2	3.3
7A	46.8 ± 0.85	13.6 ± 0.36	2.4 ± 0.16	8.15 ± 0.59	3.5 ± 0.26	1.6 ± .17	17.4 ± 1.3	25.8 ± 1.9	65.8 ± 7.1	1.8	2.6	6.7
9A	99.9 ± 2.15	17.8 ± 0.75	1.7 ± 0.13	20.96 ± 1.52	7.3 ± 0.53	1.55 ± .11	21.0 ± 1.5	41.0 ± 3.0	91.2 ± 6.5	2.15	4.2	7.6
11A	27.8 ± 0.98	3.8 ± 0.24	4.6 ± 0.21	9.6 ± 1.02	2.42 ± 0.34	2.04 ± .31	34.5 ± 3.6	63.7 ± 8.9	44.3 ± 6.7	3.5	6.3	4.6
18A	149.3 ± 2.78	33.8 ± 0.72	2.4 ± 0.10	22.96 ± 2.9	6.0 ± 0.76	0.84 ± .07	15.4 ± 2.0	17.8 ± 2.2	35.0 ± 2.9	1.86	2.1	4.2
19A	63.1 ± 1.85	14.6 ± 0.44	2.41 ± 0.13	17.4 ± 1.3	4.18 ± 0.32	1.26 ± .09	27.6 ± 2.06	28.6 ± 2.2	52.5 ± 4.6	2.9	3.0	5.4
7B	79.0 ± 1.22	22.6 ± 0.55	1.5 ± 0.12	12.2 ± 0.9	5.53 ± 0.39	1.2 ± .23	15.4 ± 1.1	24.5 ± 1.70	80.0 ± 15.3	1.5	2.4	8.0
11B	101.4 ± 2.20	24.1 ± 0.54	1.8 ± 0.11	22.16 ± 1.55	5.49 ± 0.38	1.13 ± .08	21.8 ± 1.5	22.8 ± 1.6	62.8 ± 4.4	2.17	2.24	6.1
19B	124.1 ± 2.07	31.0 ± 0.50	1.6 ± 0.09	21.14 ± 1.46	5.16 ± 0.36	0.94 ± .07	17.2 ± 1.2	16.6 ± 1.2	58.8 ± 4.4	1.66	1.6	5.6

TABLE 7

Summary of results reported in table 6

PLOT NUMBER	MEAN			σ			C.V.			Em		
	B+A	A	F	B+A	A	F	B+A	A	F	B+A	A	F
	colonies	*colonies*	*colonies*				*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
4A	47.0 ± 1.03	12.9 ± 0.35	2.0 ± 0.13	9.6 ± 0.72	3.28 ± 0.25	1.25 ± 0.09	20.4 ± 1.5	25.4 ± 1.9	62.5 ± 4.5	2.2	2.7	6.5
5A	60.6 ± 1.43	18.1 ± 0.73	3.0 ± 0.23	13.27 ± 1.01	6.85 ± 0.52	2.22 ± 0.17	21.9 ± 1.6	37.8 ± 2.9	74.0 ± 5.7	2.36	4.0	7.7
7A	36.1 ± 0.99	9.4 ± 0.26	2.0 ± 0.20	9.32 ± 0.70	2.48 ± 0.19	1.85 ± 0.13	25.8 ± 1.9	26.4 ± 2.0	92.5 ± 6.5	2.7	2.77	10.0
9A	66.5 ± 1.3	20.3 ± 0.63	1.6 ± 0.12	12.15 ± 0.91	5.95 ± 0.45	1.17 ± 0.09	18.3 ± 1.3	29.3 ± 2.2	73.1 ± 5.6	1.95	3.1	7.5
11A	15.6 ± 0.57	2.3 ± 0.16	4.3 ± 0.29	5.41 ± 0.41	1.54 ± 0.11	2.72 ± 0.21	34.7 ± 2.6	67.0 ± 4.8	63.3 ± 4.9	3.65	7.0	6.7
18A	128.0 ± 3.5	29.7 ± 0.83	3.0 ± 0.2	32.8 ± 2.5	7.76 ± 0.59	1.84 ± 0.14	25.6 ± 2.0	26.1 ± 2.0	61.3 ± 4.7	2.0	2.8	6.7
19A	40.1 ± 1.14	11.9 ± 0.49	2.0 ± 0.17	9.7 ± 0.8	4.19 ± 0.35	1.46 ± 0.12	24.2 ± 2.0	35.2 ± 2.9	69.5 ± 5.7	2.8	4.1	8.1
7B	50.9 ± 1.23	20.3 ± 0.7	1.2 ± 0.12	10.83 ± 0.88	6.13 ± 0.5	1.04 ± 0.08	21.3 ± 1.7	30.2 ± 2.4	86.7 ± 6.7	2.4	3.45	10.0
11B	77.1 ± 3.53	20.6 ± 0.67	1.3 ± 0.14	30.63 ± 2.51	5.85 ± 0.48	1.25 ± 0.10	39.7 ± 3.2	28.4 ± 2.3	96.2 ± 7.7	4.6	3.25	10.8
19B	73.0 ± 1.79	23.0 ± 0.46	1.6 ± 0.17	16.4 ± 1.26	4.22 ± 0.33	1.52 ± 0.11	22.4 ± 1.7	18.3 ± 1.4	95.0 ± 6.9	2.45	2.0	10.6

TABLE 6

*Numbers of microörganisms in the soil on June 27, 1921**

4A			5A			7A			9A			11A			18A			19A			7B			11B			19B		
B+A	A	F	B+A	A	F	B+A	A	F	B+A	A	F	B+A	A	F	B+A	A	F	B+A	A	F	B+A	A	F	B+A	A	F	B+A	A	F
42*	9	4	82	28	3	39	7	3	53	16	2	18	6	5	112	29	3	47	11	3	61	25	0	67	17	1	50	13	2
46	12	0	76	20	3	41	7	1	58	25	2	18	6	3	82	18	4	43	13	1	47	16	1	82	19	0	59	22	1
47	14	1	66	21	4	41	12	3	68	21	4	16	2	2	96	19	7	54	19	3	36	16	1	76	25	0	51	25	0
38	12	4	52	22	4	46	12	2	58	18	4	8	1	2	82	18	3	52	14	2	55	29	1	67	21	0	55	23	4
47	11	2	83	28	2	39	14	0	62	26	2	13	3	2	95	21	4	36	7	4	54	28	0	53	15	2	64	19	7
53	13	0	76	36	0	35	12	3	86	37	0	11	1	5	99	25	6	46	14	3	63	25	0	59	11	2	62	23	1
43	9	2	78	21	2	22	10	1	76	26	1	11	1	2	96	22	4	58	21	4	57	24	0	82	24	0	86	22	3
47	11	2	62	29	2	35	8	0	46	19	2	21	2	2	88	24	3				71	29	2	69	23	1	52	14	1
52	10	4	76	26	1	46	7	0	55	18	0	10	2	3	126	31	6							54	11	2	44	19	2
34	11	0				48	8	3	56	14	2	24	5	1	122	22	5							72	23	1	62	23	0
72	18	3	52	10	2	26	6	2	74	19	2	28	1	2	127	24	2	46	18	2	37	16	2	56	19	1	95	32	1
60	21	0	62	16	7	38	7	2	61	18	2	11	1	2	121	29	2	27	10	4	48	22	3	71	23	1	92	24	1
52	13	0	43	12	5	36	11	1	59	16	1	14	2	5	132	25	2	34	9	2	42	21	2	62	19	0	86	21	2
54	13	2	43	12	11	32	11	2	67	32	1	12	2	5	108	23	0	30	12	0	59	22	2	46	14	1	93	22	0
51	9	4	66	17	2	35	10	4	98	29	1	18	3	4	104	17	0	29	8	2	54	14	0	54	22	0	96	19	2
69	15	2	86	21	6	26	14	1	62	21	2	17	5	3	126	22	3	38	13	0	47	19	1	44	24	2	66	21	0
57	18	1	42	8	7	38	12	2	76	21	0	22	5	4	114	36	0	35	14	3	49	21	1	54	21	2	71	25	1
42	12	3	56	9	0	51	11	1	68	16	2	12	2	6	91	28	4	46	12	1	44	22	3	51	11	0	101	32	0
52	11	2	58	14	1	39	15	2	66	19	2	16	1	4	104	26	3							42	15	2	81	28	2
36	12	1	38	12	3	23	4	1	91	24	0	18	3	3	98	32	2										89	21	4
36	12	2	62	15	2	39	12	5	57	21	1	10	2	3	143	33	2	49	11	3	36	12	1	122	32	1	88	23	2
44	20	3	67	16	2	29	7	3	94	36	0	13	1	3	139	27	1	32	9	3	51	16	0	148	31	6	65	22	3
46	15	2	78	32	1	49	8	4	73	17	2	12	1	16	152	41	2	49	16	3	58	26	0	136	28	3	61	26	0
40	8	3	52	12	1	26	7	0	52	18	3	21	2	3	138	28	1	57	24	0	57	19	0	117	26	0	69	28	1

---

* Cf. footnote to table 4, p. 326.

42	12	2	65	14	3	46	12	6	75	22	1	17	2	7	106	29	2	34	8	4	46	17	3	92	21	1	59	23	1
45	14	3	58	20	0	22	5	2	52	18	1	21	3	5	136	33	2	51	14	2	82	35	2	136	32	3	65	31	2
42	8	2	54	14	5	49	9	1	58	27	3	9	1	8	123	27	2	41	11	2	39	15	1	108	23	1	44	21	2
44	12	4	42	8	2	61	14	9	64	19	1	31	4	4	96	26	3	42	13	2	51	17	2	128	24	3	67	28	0
41	15	1	73	24	4	29	11	0	89	18	2	16	1	3	93	29	3				49	22	0				76	26	4
52	14	2	61	17	1	39	10	1	62	29	0	19	3	8							63	36	0						
46	14	1	57	14	4	43	12	1	68	15	1	13	3	3	185	45	0	29	8	1	44	13	2	77	18	2	86	26	3
51	16	0	62	16	4	35	11	1	64	17	2	12	3	7	161	36	4	49	12	0	38	14	1	66	18	1	88	21	0
46	10	2	67	23	4	38	8	0	67	13	2	12	0	5	158	42	7	46	11	0	41	16	2	72	16	2	85	27	0
61	16	1	32	7	3	29	8	0	58	16	2	13	2	6	168	36	3	33	6	2	44	11	2	81	23	1	103	24	0
33	7	4	46	16	6	39	10	2	53	13	0	19	4	10	190	44	2	38	14	0	43	15	2	56	13	2	83	20	3
56	17	3	60	17	2	24	10	2	61	16	2	12	1	3	178	39	2	32	8	2	38	15	0	74	25	0	68	22	1
32	9	2	61	17	2	21	9	4	64	16	4	6	0	5	186	38	6	26	8	5	56	24	1	47	12	1	62	19	2
38	16	3	48	21	3	29	6	3	76	16	0	20	1	6	174	40	5	44	11	1	52	20	0				71	21	1
29	12	2	53	16	2	24	8	0	62	13	2	9	2	4	182	42	2	30	9	0	71	19	3				79	18	2
62	15	2	68	25	2	38	7	2	72	18	4	19	1	5	162	31	4	21	6	4									

TABLE 8

*Numbers of microörganisms in the soil on July 18, 1921**

4A		5A		7A		9A		11A		18A		19A		7B		11B		19B	
B+A	A	B+A	A	B+A	A	B+A	A	B+A	A	B+A	A	B+A	A	B+A	A	B+A	A	B+A	A
85*	23	114	31	41	7	132	26	35	1	141	36	38	11	71	24	91	22	76	15
84	21	122	24	27	6	108	24	16	2	106	39	47	18	51	12	123	29	74	21
66	22	99	39	46	11	102	17	24	2	103	33	55	19	74	23	97	23	93	32
86	24	153	48	32	11	143	31	23	1	81	18	36	6	52	14	102	19	66	19
84	30	111	38	28	12	121	36	18	2	148	42	28	7	68	21	114	26	67	24
75	18	118	42	34	13	116	32	26	2	119	32	61	19	96	26	95	28	65	18
76	25	109	24	30	10	105	25	21	1	110	24	47	11	58	14	84	18	76	22
74	23	88	36	28	5	129	17	33	1	139	29	51	13	66	22	118	24	59	14
82	28	133	38	27	7	134	28	17	2	97	34			71	31			77	20
58	16	97	24	41	13	122	32							67	22			63	23
44	19	106	32	38	12	96	24	22	0	117	36	35	14	74	21	79	30	90	21
67	24	89	28	22	8	97	22	27	2	134	43	22	8	78	23	66	22	82	17
79	27	93	25	29	6	99	27	12	0	91	25	43	12	88	23	84	27	72	14
51	18	102	27	24	11	113	23	24	1	99	34	51	14	76	26	98	24	81	27
65	23	86	26	46	9	112	22	27	2	73	12	53	18	60	22	76	24	64	21
41	12	92	14	36	8	107	32	26	1	93	37	35	17	61	24	81	19	91	26
72	22	96	21	44	14	106	24	25	2	77	19	37	14	68	16	58	18	66	25
44	11	82	26	41	7	106	23			82	18	31	16	64	23	104	29	62	18
48	12	84	24	42	14	132	42			97	16			63	17			53	17
43	10	98	26	31	11	98	25			127	31			49	24			67	15
75	23	81	28	31	14	106	28	21	2	131	34	45	7	79	26	88	19	77	21
48	13	142	34	30	9	118	27	41	1	98	26	48	14	69	18	98	22	58	18
79	27	153	32	26	8	131	21	37	0	102	32	58	17	48	17	119	26	98	17
56	15	139	46	24	8	85	22	23	0	118	35	31	12	56	18	107	28	73	26

---

* Cf. footnote to table 4.

42	14	116	38	32	6	148	46	30	2	107	36	45	12	62	16	102	21	94	21
53	13	92	22	27	6	79	23	33	1	83	23	51	11	57	20	115	29	64	20
44	14	104	30	29	8	117	23	49	1	73	19	41	18	76	23	81	26	59	18
41	11	143	38	33	9	98	22	42	1	129	27	57	13	59	18	92	21	71	20
53	15	117	37	32	8	124	24					54	15	61	19	126	32		
49	13	94	27	37	7	82	18							65	21	118	24		
68	19	110	31	45	11	92	17	38	4	102	18	48	16	69	17	87	26	102	26
76	18	83	32	46	11	102	24	19	2	124	17	49	14	109	27	61	22	71	19
67	21	97	28	48	9	106	19	17	1	105	24	55	15	116	31	79	23	96	26
98	20	92	33	48	16	104	18	31	1	109	31	49	14	89	21	81	27	69	27
61	11	138	41	53	13	83	18	33	3	103	27	39	8	92	23	102	35	84	27
86	16	115	28	39	12	89	16	18	2	121	29	34	7	81	32	73	18	95	29
89	14	131	34	31	9	114	18	26	4	89	21	56	14	112	33	61	18	101	28
56	15	130	39	49	13	84	14	34	5	91	34	68	12	72	22	72	27	78	26
82	21	112	28	52	14	94	13			76	24			63	11				
101	19	122	36	44	9					90	26								
86	22	128	32	31	9	167	28	59	9	112	33	34	8	104	28	69	19	66	17
91	19	98	19	29	5	182	51	24	2	137	32	50	22	110	42	97	16	68	21
102	26	92	18	37	9	143	35	28	1	125	21	56	13	101	33	94	24	45	13
76	16	162	23	34	6	124	28	27	2	138	34	48	14	82	26	82	15	66	17
98	17	79	16	38	6	98	24	37	1	112	32	45	14	68	27	112	28	71	19
69	11	103	16	29	8	155	32	22	1	102	23	47	17	94	29	73	18	69	18
73	12	122	38	19	5	138	25	43	5	91	14	33	11	71	31	92	26	72	19
91	18	118	26	46	12	162	27	38	3	106	28	34	12	72	22	115	32	49	17
76	18	131	36	42	9	170	38	18	1	131	29			67	19	77	20		
72	16	137	34	16	6			33	2	127	35					109	28		

TABLE 10

*Numbers of microörganisms in the soil on September 22, 1921**

4A		5A		7A		9A		11A		18A		19A		7B		11B		19B	
B+A	A	B+A	A	B+A	A	B+A	A	B+A	A	B+A	A	B+A	A	B+A	A	B+A	A	B+A	A
77*	28	154	32	44	14	134	36	47	4	145	32	58	14	131	25	98	31	170	59
101	26	156	38	36	15	152	54	57	7	148	48	46	9	108	26	121	39	183	52
102	28	124	30	39	12	114	57	55	6	170	61	54	11	117	29	124	41	142	54
93	32	167	51	36	8	100	42	44	5	146	44	42	7	105	31	114	43	170	56
78	24	164	57	57	23	98	26	51	7	158	42	55	13	111	23	148	42	169	66
111	22	108	27	29	9	122	49	43	13	153	46	49	12	106	34	137	36	173	53
69	18	149	43	54	19	108	28	39	4	157	71	48	12	113	31	129	34	137	49
83	27	138	40	69	19	113	37	42	8	163	53	62	11	101	29	133	32	142	44
102	20	214	70	51	14	126	38	44	3	166	52	74	16	104	32	109	31	113	25
82	18	203	44	39	7	104	40	37	1	238	59	66	12	99	29	73	23	98	29
59	13	184	36	67	16	114	44	28	3	169	45	62	13	86	27	132	44	158	37
110	24	182	37	46	12	106	32	55	4	246	72	97	21	89	26	128	39	112	31
100	20	224	56	41	15	132	42	52	5	253	82	81	10	101	34	113	43	176	45
52	12	193	74	66	20	110	42	61	4	186	46	53	12	92	29	79	24	124	37
102	26	178	62	43	12	112	39	32	2	234	71	59	14	123	36	117	29	114	28
55	11	197	56	56	13	103	35	42	6	207	58	63	13	78	37	122	34	106	29
65	16	222	53	57	14	139	28	59	4	136	37	52	10	98	39	103	28	153	34
58	17	186	38	64	17	174	47	54	8	147	38	59	14	79	26	98	26	141	39
69	19	227	49	53	12	193	34	74	8	144	42	62	18	92	51	89	33	146	36
71	23	178	41	57	18	172	52	32	2	138	44	108	22	92	29	81	24	162	45
49	10	230	52	33	11	146	32	29	2	190	56	55	16	102	47	123	37	132	37
37	12	256	68	28	15	118	32	44	5	152	42	69	13	89	33	71	23	149	42
79	19	218	67	46	12	114	41	37	3	212	68	77	14	103	42	88	33	127	43
60	14	168	34	39	7	153	46	49	4	141	46	83	16	104	43	112	31	144	42

* Cf. footnote to table 4, p. 326.

106	28	136	37	33	6	136	38	63	9	145	36	66	13	101	26	130	38	142	41
70	19	178	46	44	13	92	22	66	14	192	48	72	16	112	27	123	41	149	43
66	15	169	49	66	13	102	26	77	24	138	41	63	14	139	31	126	36	106	29
59	15	168	42	49	17	130	38	41	8	147	43	91	19	99	24	128	37	144	34
78	23	197	45	46	18	98	26	55	12	134	40	58	13	109	31	111	29	89	28
73	22	194	46	49	13	74	18	86	10	204	49	73	14	112	31	121	41	137	36
76	26	192	48	68	19	111	27	44	8	223	58	69	12	82	29	92	26	112	31
91	21	184	42	65	23	83	25	49	7	118	37	64	14	77	26	99	28	124	29
84	31	124	30	38	9	129	36	61	7	116	24	68	21	104	19	98	28	137	34
61	20	152	38	43	16	106	33	48	4	159	34	64	11	108	26	88	19	99	29
102	23	130	46	47	12	97	18	49	9	133	30	56	14	103	24	124	26	151	42
89	22	104	28	48	14	110	45	38	6	146	37	63	19	92	29	92	28	133	29
83	23	164	49	34	12	168	43	51	6	152	43	49	14	99	28	66	21	112	28
63	18	128	31	42	7	132	52	79	16	138	29	67	18	139	31	95	28	81	33
68	16	126	32	39	6	153	57	41	6	155	39	78	17	109	28	113	35	123	28
72	19	143	39	41	8	129	41	62	6	182	48	83	18	88	26	137	39	92	31

TABLE 9

Summary of results reported in table 8

PLOT NUMBER	MEAN		σ		C.V.		Em	
	B+A	A	B+A	A	B+A	A	B+A	A
	colonies	*colonies*			*per cent*	*per cent*	*per cent*	*per cent*
4A	69.6 ± 1.67	18.3 ± 0.49	17.56 ± 1.18	5.14 ± 0.35	25.2 ± 1.7	28.1 ± 1.9	2.4	2.7
5A	111.1 ± 2.02	30.3 ± 0.74	21.25 ± 1.43	7.74 ± 0.52	19.1 ± 1.3	25.5 ± 1.7	1.8	2.4
7A	35.3 ± 0.84	9.4 ± 0.27	8.83 ± 0.59	2.86 ± 0.19	25.0 ± 1.7	30.4 ± 2.0	2.4	2.9
9A	116.1 ± 2.4	25.7 ± 0.86	24.73 ± 1.7	7.85 ± 0.54	21.3 ± 1.5	30.5 ± 2.1	2.07	3.3
11A	28.5 ± 1.0	2.0 ± 0.17	9.7 ± 0.7	1.63 ± 0.12	34.0 ± 2.4	81.5 ± 6.0	3.5	8.5
18A	105.5 ± 1.97	27.5 ± 0.74	20 0 ± 1.38	7.57 ± 0.49	18.9 ± 1.3	27.5 ± 1.8	1.9	2.7
19A	45.0 ± 1.06	13.3 ± 0.39	10.05 ± 0.76	3.74 ± 0.28	22.3 ± 1.7	28.1 ± 2.1	2.35	2.9
7B	74.1 ± 1.7	22.9 ± 0.58	17.53 ± 1.2	6.08 ± 0.42	23.7 ± 1.6	26.5 ± 1.8	2.3	2.5
11B	92.1 ± 1.81	23.5 ± 0.48	17.76 ± 1.27	4.71 ± 0.33	19.3 ± 1.4	20.0 ± 1.4	1.96	2.04
19B	73.6 ± 1.4	20.4 ± 0.47	13.81 ± 0.99	4.58 ± 0.33	18.8 ± 1.3	22.4 ± 1.6	1.9	2.3

TABLE 11

Summary of results reported in table 10

PLOT NUMBER	MEAN		σ		C.V.		Em	
	B+A	A	B+A	A	B+A	A	B+A	A
	colonies	*colonies*			*per cent*	*per cent*	*per cent*	*per cent*
4A	75.7 ± 1.99	20.5 ± 0.58	18.7 ± 1.4	5.5 ± 0.61	24.7 ± 1.8	26.8 ± 2.0	2.63	2.83
5A	172.7 ± 3.8	45.1 ± 1.26	35.6 ± 2.7	11.9 ± 0.9	20.6 ± 1.6	26.4 ± 2.0	2.2	2.8
7A	47.6 ± 1.22	13.5 ± 0.47	11.46 ± 0.87	4.42 ± 0.33	24.1 ± 1.8	32.7 ± 2.4	2.56	3.48
9A	122.7 ± 2.77	37.5 ± 1.06	25.98 ± 1.96	10.0 ± 0.75	21.2 ± 1.6	26.7 ± 2.0	2.26	2.83
11A	50.4 ± 1.44	6.8 ± 0.32	13.54 ± 1.02	4.35 ± 0.33	26.9 ± 2.0	64.0 ± 4.6	2.86	4.7
18A	167.0 ± 3.77	47.3 ± 1.37	35.36 ± 2.68	12.9 ± 0.98	21.2 ± 1.6	27.3 ± 2.1	2.26	2.9
19A	65.5 ± 1.5	13.8 ± 0.36	14.0 ± 1.06	3.41 ± 0.26	21.4 ± 1.6	24.7 ± 1.9	2.3	2.6
7B	102.4 ± 1.55	30.6 ± 0.69	14.5 ± 1.1	6.5 ± 0.49	14.2 ± 1.1	21.7 ± 1.6	1.54	2.25
11B	109.6 ± 2.17	32.5 ± 0.69	20.44 ± 1.55	6.54 ± 0.48	18.7 ± 1.4	20.1 ± 1.5	2.0	2.1
19B	134.3 ± 2.75	38.4 ± 1.06	25.77 ± 2.01	9.95 ± 0.75	19.1 ± 1.5	25.9 ± 1.9	2.05	2.76

TABLE 12

Numbers of fungi in the soil on July 18 and September 22, 1921, as determined on special acid

PLOT NUMBER	NUMBER OF FUNGI PER GRAM	
	July 18	Sept. 22
4A	31,000	45,600
5A	54,000	91,000
7A	56,000	63,000
9A	47,000	45,900
11A	115,000	107,900
18A	73,000	87,600
19A	61,000	48,200
7B	28,000	16,900
11B	44,000	34,200
19B	32,000	26,200

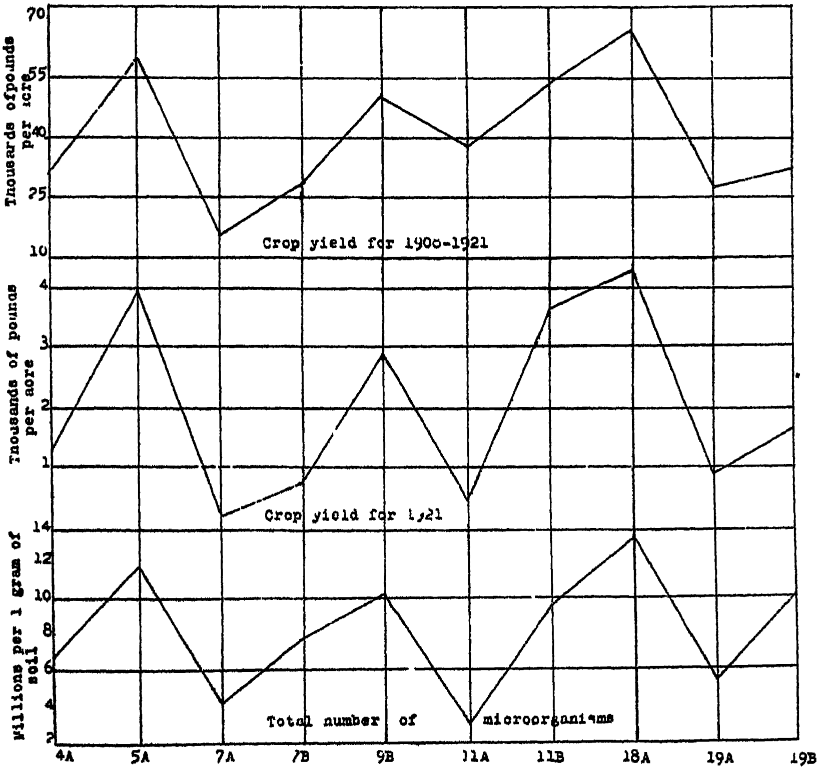


FIG. 2. CROP YIELDS FROM PLOTS AND BACTERIAL NUMBERS

Figures for yields designate thousands of pounds of dry matter per acre; the numbers of microörganisms are in millions per gram of moist soil.

TABLE 13

Summary of results—Average of 4 samplings

PLOT NUMBER	B + A	A	F*	F†	AVERAGE MOISTURE	AVERAGE REACTION	SOIL TREATMENT
	number	*number*	*number*	*number*	*per cent*	*pH*	
4A	7,490,000	920,000	264,000	31,000	15.0	5.8	Minerals only
	4,700,000	1,290,000	203,000	45,600	5.3	5.4	
	6,960,000	1,830,000			12.3	5.5	
	7,570,000	2,050,000			13.8	5.7	
Aver......	6,680,000	1,520,000	234,000	38,300			
$\frac{Aver.}{B + A}$		*22.7%*	*3.5%*				
5A	12,450,000	2,320,000	365,000	54,000	10.0	5.6	Manure
	6,060,000	1,810,000	303,000	91,000	4.8	5.0	
	11,110,000	3,030,000			12.5	5.4	
	17,270,000	4,510,000			11.7	5.5	
Aver......	11,720,000	2,920,000	334,000	72,500			
$\frac{Aver.}{B + A}$		*24.5%*	*2.9%*				
7A	4,680,000	1,360,000	240,000	56,000	12.0	4.0	Control, no fertilizer
	3,610,000	940,000	200,000	63,500	5.3	4.0	
	3,530,000	940,000			8.2	5.1	
	4,760,000	1,350,000			10.7	5.1	
Aver......	4,150,000	1,150,000	220,000	59,750			
$\frac{Aver.}{B + A}$		*27.7%*	*5.3%*				
9A	9,990,000	1,780,000	170,000	47,000	10.0	5.8	$NaNO_3$ + minerals
	6,650,000	2,030,000	163,000	45,900	3.7	5.3	
	11,610,000	2,570,000			12.6	5.4	
	12,270,000	3,750,000			12.5	5.6	
Aver......	10,130,000	2,530,000	167,000	46,450			
$\frac{Aver.}{B + A}$		*25.0%*	*1.65%*				
11A	2,780,000	380,000	457,000	115,000	12.0	3.9	$(NH_4)_2SO_4$ + minerals
	1,560,000	230,000	433,000	107,900	4.8	3.9	
	2,850,000	200,000			11.4	3.9	
	5,040,000	680,000			12.6	4.7	
Aver......	3,060,000	370,000	445,000	111,450			
$\frac{Aver.}{B + A}$		*12.1%*	*14.5%*				

* Results based on tables 4 and 6.

† Results based on table 12.

TABLE 13—*Continued*

PLOT NUMBER	B + A	A	F	F	AVERAGE MOISTURE	AVERAGE REACTION	SOIL TREATMENT
	number	*number*	*number*	*number*	*per cent*	*pH*	
18A	14,930,000	3,380,000	235,000	73,000	10.0	5.8	Manure + $NaNO_3$ + minerals
	12,800,000	2,970,000	300,000	87,600	5.0	5.2	
	10,550,000	2,750,000			12.1	5.3	
	16,700,000	4,730,000			11.4	5.6	
Aver......	13,750,000	3,460,000	268,000	80,300			
$\frac{\textit{Aver.}}{B + A}$		*25.2%*	*1.95%*				
19A	6,310,000	1,460,000	243,000	61,000	10.0	5.4	Minerals only
	4,010,000	1,190,000	206,000		3.6	5.0	
	4,500,000	1,330,000			9.8	5.0	
	6,550,000	1,380,000			11.2	5.4	
Aver......	5,340,000	1,340,000	225,000	61,000			
$\frac{\textit{Aver.}}{B + A}$		*25.1%*	*4.2%*				
5B‡	22,000,000			29,400	12.0	7.0	Manure + minerals + lime
7B	7,720,000	2,260,000	146,000	28,000	10.0	6.4	Lime only
	5,090,000	2,030,000	114,000	16,900	3.1	6.2	
	7,410,000	2,290,000			9.8	6.8	
	10,240,000	3,060,000			11.4	6.2	
Aver......	7,620,000	2,410,000	130,000	22,450			
$\frac{\textit{Aver.}}{B + A}$		*31.6%*	*1.7%*				
11B	10,140,000	2,410,000	180,000	44,000	10.0	6.0	$(NH_4)_2SO_4$ + minerals + lime
	7,710,000	2,000,000	130,000	34,200	3.1	5.8	
	9,210,000	2,350,000			9.9	6.0	
	10,960,000	3,250,000			9.0	5.5	
Aver......	9,510,000	2,520,000	155,000	39,100			
$\frac{\textit{Aver.}}{B + A}$		*26.5%*	*1.63%*				
19B	12,400,000	3,100,000	164,000	26,200	10.0	6.6	Minerals + lime
	7,300,000	2,300,000	163,000		3.4	6.8	
	7,360,000	2,040,000			9.8	7.0	
	13,430,000	3,840,000			10.1	6.5	
Aver......	10,120,000	2,820,000	164,000	26,200			
$\frac{\textit{Aver.}}{B + A}$		*27.8%*	*1.6%*				

---

‡ This plot had the same treatment as 5A with the addition of lime; it had been sampled but once (Sept. 22), and the results are given here for comparison.

column represents the colonies obtained from one particular plot, at one particular date, with 100,000 dilution or 1000 dilution for special acid medium (table 12). Each group in the column represents the plates counted for each of the different composite samples taken from each plot. The results are calculated by the use of the formulae given in Paper I of this series (16), namely:

$$\text{Average deviation}, \epsilon = \sqrt{\frac{\Sigma\,(Zn - a)^2}{n - 1}}$$

$$\text{Most probable error} = \pm\,0.6745\sqrt{\frac{\Sigma\,(Zn - a)^2}{n(n - 1)}}$$

$$C.V. = \frac{\text{the average deviation} \times 100}{\text{mean}}$$

$$Em = \frac{\text{most probable error} \times 100}{}$$

DISCUSSION OF RESULTS

To be able to discuss the results more thoroughly, all the tables have been condensed into table 13 and figure 2 which show at a glance the influence of fertilization upon the numbers of the different groups of microörganisms in the soil, as determined by the plate method.

The lowest number of microörganisms is found in the plot receiving annually ammonium sulfate without any application of lime. This plot has become so acid that there was practically no crop during the summer in question; there was, however, a second crop consisting chiefly of crab grass. This ammonium sulfate plot gave the lowest number of microörganisms, since the reaction of the soil during the major part of the summer was less than pH 4.0, which is a reaction distinctly unfavorable to bacterial activities. The actinomycetes were even more affected than the bacteria, so that not only did the total number of microörganisms (except the fungi) drop down to 3 million per gram, but the percentage of the actinomycetes was only 12.1, while in the untreated plot it was 27.7. This is due to the fact that the actinomycetes, as shown by the writer elsewhere (18), are very sensitive to acid conditions. However the acid reaction did not prevent the growth of the fungi, which developed even to a greater extent due to the presence of a good source of nitrogen and probably due to the lack of competition from the bacteria and actinomycetes. There were 111, 450 fungi per gram of soil, in this plot, using the acid medium, a greater number than that of any other plot. This was equal to 14.5 per cent of the number of bacteria and actinomycetes on the bacterial plate. In this plot we find a striking correlation between a low crop yield (practically a minimum) and a low number of microörganisms. The low number of actinomycetes and high number of fungi are rather a result of the reaction of the plot than of any lack in fertility. In the study of this plot, the correlation found is between bacterial activities, as expressed in numbers of microörganisms, and the chemial condition in the soil, rather than its fertility.

As soon as lime is added to the soil and the acidity resulting from the use of ammonium sulfate is neutralized, there is a rise both in crop productivity and bacterial numbers. This reverse picture is found in plot 11B which received, in addition to an equivalent amount of ammonium sulfate and minerals as 11A, also limestone at the rate of 2 tons per acre every 5 years. The productivity of this plot is very high, giving throughout the whole period as good yields as the sodium nitrate plot (9A), but smaller yields than the two manured plots (5A and 18A). The number of microörganisms on this plot are only a trifle less than those on the nitrate plot and appreciably less than in the two manured plots. The percentage of actinomycetes increased from 12.1 to 26.5, or from the lowest of the whole series to the general average, while the fungi dropped from 111,450 to 39,100 per gram on the acid media and from 14.5 to 1.63 per cent on the general plates. This bears out again the statement made in the previous paragraph that the total and relative numbers of actinomycetes and fungi are a function of the soil reaction rather than of the chemical and bacteriological condition of the soil. The correlation between the productivity of the soil and total number of microörganisms is again brought out.

Plot 9A which received annually sodium nitrate behaved in general like 11B, as seen from the following correlation:

	PLOT 9A	PLOT 11B
Total bacteria	10,113,000	9,500,000
Actinomycetes, per cent	25	26.5
Fungi (acid medium)	46,450	39,100
Fungus ratio on general plate	1.65	1.63
Reaction, pH	5.5	5.8
Total crop yield, lbs.	50,488	53,826

When the fact is kept in mind that the crop yields are quite similar on the two plots (slight differences may be due to preference of plants in question for the particular source of nitrogen or for calcium as a fertilizer), we may conclude that the two fertilizers affect alike the numbers of microörganisms in the soil, as long as there is enough lime in the soil to neutralize the acids left or formed from the ammonium sulfate. Again the bacterial numbers in the soil are found to be a function of the productivity of the soil, when properly determined and when the variability is accounted for.

When the two plots 9A and 11B are compared with plots 4A and 19A which received the same amount of minerals but no nitrogenous fertilizer, we find that the addition of the nitrogenous fertilizer, where it did not result in an acid reaction, as in 11A, resulted in an increase both of crop production and numbers of microörganisms. It is interesting to note that, while the yield from 9A is nearly double the yield obtained by averaging 4A and 19A, the numbers of microörganisms are also nearly double. While the ratio of actinomycetes remained the same, the ratio of the fungi to the total number of microörganisms is 3.7 per cent for the plots without any nitrogenous fertilizer and 1.64 per cent for the two fertilized plots (9A and 11B). This may be due either to a slight difference in reaction, which is somewhat more acid in the former, or to a stimulating effect of the nitrogenous fertilizers upon the activities of bacteria and actinomycetes to a much greater extent than of the fungi.

If lime is added to the soil in addition to minerals (plot 19B) there is a decided increase in the number of microörganisms. The reaction of all the plots not receiving lime is decidedly acid and we may assume that the activities of microorganisms in these plots are kept somewhat in check. The soil in 11B receiving lime is still decidedly acid due to the large amounts of ammonium sulfate used. In 19B, however, the addition of lime resulted in making the soil neutral. This resulted in an increase in the ratio of actinomycetes, a decided decrease both in the numbers and ratio of fungi and an increase in the total numbers of microorganisms (bacteria + actinomycetes). This is accompanied by an increase in crop yield so that the crop obtained from 19B is almost double, for 1921 than that of 19A. If there is no close parallel as in the case of the previous plots, it is no doubt due to the lack of uniformity of the soil in the different plots as seen from 4A and 19A which, although receiving the same treatment, gave different crop yields during the different periods.

Taking up the two manured plots, one without (5A) and one (18A) with sodium nitrate, we obtain the greatest number of microörganisms. The fact that manure greatly stimulates an increase in bacterial numbers has been brought out by various investigators, Engberding (2), Temple (15), and others. The numbers of actinomycetes increased with the bacteria so that the same ratio is obtained. As to the fungi, there is a definite increase in numbers in both plots as shown by the use of the special acid medium, but the ratio of fungi to the total number was decreased somewhat, more so in the plot that received also sodium nitrate. This would indicate that the addition of manure to the soil results in an increase in the numbers of fungi, but not to such an extent as do the bacteria and actinomycetes, which results in a drop in the ratio of fungi. In the case of these two manured plots, we find again a definite correlation between the soil productivity, as indicated by the crop yield for the whole period, and numbers of microörganisms. The plot (5B) that received both manure and lime was sampled only once (Sept. 22). It indicated a further increase in the total number of microörganisms (22,000,000 as compared with 17,270,000 in 5A at the period of sampling) and a drop in the fungus numbers which would be expected from the change of reaction to neutrality. This was also accompanied by a greater crop yield.

The two plots receiving no mineral or nitrogenous fertilizers at all are 7A and 7B, the latter being the limed plot. The first plot has become so exhausted due to continuous cropping without fertilizer that the crop of hay the year under study, was almost negligible. This may be due either to the high acidity of the plot, particularly in the early part of the summer, to the exhaustion of nutrients or accumulation of toxic products. Whatever that may be, the crop yield was paralleled by the number of microörganisms. With the exception of 11A, 7A gave the lowest numbers of microörganisms, including both bacteria and actinomycetes. The ratio of the latter was not so low as in 11A, but remained normal, while the number and ratio of the fungi was much above normal (in comparison with plots 4A or 19A), although less than in 11A. This would indicate that the conditions that brought about the lack of fertility in plot 7A, due to continued cultivation, injured bacteria and actinomycetes alike, but not the fungi. If it is a question of reaction, which may be quite probable, it is easily explained by the fact that the acid reaction favors the development of fungi in preference to the bacteria and actinomycetes. However, the latter organisms are not injured to such an extent as in Plot 11A, possibly due to the fact that the organic acids formed in 7A and which account chiefly for the acidity of this plot are less injurious to actinomycetes than the inorganic acids resulting from the application of ammonium sulfate in 11A. As a matter of fact, the actinomycetes can use organic acids as sources of carbon and break them down thus tending to make the medium less acid.

The addition of lime to the plot receiving no fertilizer (7B) resulted in an increase in the numbers of microörganisms, an increase in the ratio of actinomycetes, a decrease in the ratio and numbers of fungi, and an increase in crop production.

It would therefore seem conclusive, from the above considerations, that the total number of microörganisms as determined by the plate method can serve as a function of the bacteriological condition of the soil and as an index of soil fertility. The relative numbers of bacteria, actinomycetes and fungi indicate also the chemical condition of the soil.

Manure has the greatest effect in stimulating the numbers of microörganisms in the soil. Next to the action of manure, liming of acid soils stimulates the development of bacteria and actinomycetes. While manure offers a source of energy for the activities of bacteria, fungi and actinomycetes, lime neutralizes the soil acidity making the soil a more favorable medium for the development of bacteria and even more so for actinomycetes, while the fungi are depressed either due to the unfavorable effect of the reaction or to the competition of the other microörganisms (antagonistic effects included). The addition of lime stimulating the development of bacteria and actinomycetes, which are able to decompose the soil organic matter, thus making the nitrogen stored away available for higher plants, is equivalent to nitrogen fertilization. The addition of lime to the soil is found to have a more stimulating effect upon the number of microörganisms than upon crop yield, as shown in Fig. 2 (7B and 19B). This is due to two or more factors. The addition of lime in the above cases brought about a change in reaction critical to the growth of various bacteria, like Azotobacter, and actinomycetes; the plants which may not be so sensitive to the acid reaction of the soil as certain groups of bacteria are probably not affected as favorably as those bacteria. The addition of lime to an acid soil also brings about a redistribution of the various groups of soil microörganisms (qualitative) which may account for the lack of perfect parallelism.

Artificial fertilizers serve as direct plant food for higher plants and stimulate the activities of microörganisms, so far as those developing on the plate are concerned, only to a smaller extent. They modify largely the activities of microörganisms in the soil due to the secondary reaction produced, such as change in reaction or physical condition of soil, etc., which will allow specific groups of microörganisms to develop.

When the different periods of sampling are compared, we find that, at the end of dry spells, as in the case of the second sampling, the numbers of microorganisms drop down. The question then arises, when is the proper time to sample the soil, so as to obtain a representative bacterial flora? If the soil is sampled after a long dry period, the numbers will be much below the average, the drop not taking place alike in all soils. As a simple example, we may cite the fact that although in 5A, the numbers dropped from the May to the June sampling more than one-half, then increased to nearly three times in September, in plot 7A they dropped only one-quarter and then increased again by one-third. This is due to two causes: the conditions in 7A are unfavorable for bacterial activities and the forms present are more or less resistant, such as spore formers, etc., while 5A offers good condition for bacterial development. A prolonged dry period therefore, is bound to have a much greater effect on the bacteria in 5A than in 7A. Then again when the soil becomes again moist, the abundant organic matter in 5A will allow a rapid development of the bacteria, while in 7A it will take place only to a limited extent.

This brings us to the second point: should the soils be sampled shortly after a heavy rainfall? In this case the reverse conditions will hold true with results more favorable for the optimum soil conditions, as pointed out previously. The prolonged drying followed by heavy rainfall will act as a rapid shift in bacteriological equilibrium (see table 1), which will not be manifested alike in poor and fertile soils. The most ideal time for sampling would be not after a dry period and not soon after a wet period, but somewhere in between. However, before we have more definite information on the change of bacterial equilibrium in the soil, we have to take several samples during the growing season and base our results upon these.

SUMMARY

The results obtained from the investigations presented in this and the previous two papers dealing with the numbers of microörganisms in the soil can be summarized as follows:

1. The results based on one soil sample only are valueless. Several samples of the same soil, composite, if possible, should be used.

2. A large enough number of plates should be used (8–10 for each soil sample) so that the most probable error of the mean for the total number of micro-

organisms in each soil be less than 5 per cent. When it is reduced to 2 or 2.5 per cent the results are more valuable.

3. Only the numbers of bacteria and actinomycetes should be determined on the regular plate. Special acid media should be used (pH = 4.0), with a dilution one-hundredth of that used for the determination of the total number of microörganisms, for the determination of numbers of fungi in the soil.

4. Synthetic media should be used. The plates should be incubated at 25–28°C. for 7 days, or at room temperature for 14 days. All colonies should be counted on the plate, with an optimum of 50—200 colonies of microörganisms per plate. The only plates discarded from the count are those badly overgrown with fungi.

5. With these precautions carefully observed, it has been found that fertilizer treatment exerted the following influences upon the numbers of microorganisms in the soil, using a sandy loam, not very rich in organic matter:

(*a*) Potassium salt and phosphates stimulated the development of microorganisms, more so in the presence of lime than in its absence.

(*b*) The addition of lime resulted in a decrease in the numbers of fungi and an increase in numbers of bacteria and actinomycetes.

(*c*) Sodium nitrate stimulated the development of bacteria and actinomycetes but not of fungi. Ammonium sulfate, making the soil distinctly acid, stimulated the development of fungi, with a decided decrease in the numbers of bacteria and particularly actinomycetes. Where lime was used together with the ammonium sulfate, the stimulating influence was equal to that of sodium nitrate.

(*d*) Manure exerted a decided stimulating effect upon all groups of microorganisms developing on the plate.

6. Crop production ran nearly parallel, in the particular soil, with the numbers of microörganisms in the soil developing on the plate.

CONCLUSION

The numbers of microorganisms in the soil, when determinations are carried out under proper conditions, with due allowance for the variability of the methods used and soils, can serve as one function for measuring the bacteriological condition of the soil and crop production. To determine the bacteriological condition properly, we must find other functions, which will be the subject of the following investigations.

REFERENCES

(1) Clark, W. M., and Lubs, H. A. 1917 The colorimetric determination of the hydrogen-ion concentration and its applications in bacteriology. *In* J. Bact. v. 2, p. 1–34, 109–136, 191–236.

(2) Engberding, D. 1909 Vergleichende Untersuchungen über die Bakterienzahl im Ackerboden in ihrer Abhängigkeit von äusseren Einflussen. *In* Centbl. Bakt. (etc.), Abt. II, v. 23, p. 569–642.

(3) Fabricius, O., and v. Feilitzen, H. 1905. Ueber den Gehalt an Bakterien in jungfräulichen und kultivierten Hochmoorboden auf dem Versuchsfelde des Schwedischen Moorkulturvereins bei Flahult. *In* Centbl. Bakt. (etc.), Abt. II, v. 14, p. 161–168.

(4) Fischer, H. 1909 Besitzen wir eine brauchbare Methode der bakteriologischen Bodenuntersuchung? *In* Centbl. Bakt. (etc.) Abt. II, v. 23, p. 144–159.

(5) Gillespie, L. J. The reaction of soil and measurements of hydrogen-ion concentration. *In* J. Wash. Acad. Sci., v. 6, p. 7–16.

(6) Heinze, B. 1920 Bakteriologische Versuche. *In* Landw. Jahrb., v. 55, p. 139–184.

(7) Hiltner, L., und Störmer, K. 1903 Studien über die Bakterienflora des Ackerbodens, mit besonderer Berücksichtigung ihres Verhaltens nach einer Behandlung mit Schwefel kohlenstoff und nach Brache. *In* Arb. Biol. Abt. Land u. Forstwirt. K. Gesundheitsamt. 3, p. 445–545.

(8) Krüger, W., and Heinze, B. 1907 Untersuchungen über das Wesen der Brache. *In* Landw. Jahrb., v. 36, p. 383–423.

(9) Lipman, J. G., and Blair, A. W. 1913 Field experiments on the availability of nitrogenous fertilizers. N. J. Agr. Exp. Sta. Bul. 260, p. 33.

(10) Lipman, J. G., and Blair, A. W. 1920 Field experiments on the availability of nitrogenous fertilizers, 1908–1917. *In* Soil Sci., v. 9, p. 371–392.

(11) Löhnis, F. 1910 Handbuch der landwirtschaftlichen Bakteriologie. Borntraeger. Berlin.

(12) Neller, J. R. 1920 The oxidizing power of soil from limed and unlimed plots and its relation to other factors. *In* Soil Sci., v. 10, p. 29–39.

(13) Noyes, H. A., and Conner, S. D. 1919 Nitrates, nitrification, and bacterial contents of five typical soils as affected by lime, fertilizer, crops and moisture. *In* Jour. Agr. Res., v. 16, p. 27–42.

(14) Rahn, O. 1908. Bakteriologische Untersuchungen über das Trocknen des Bodens. *In* Centrbl. Bakt. (etc.) Abt. II, v. 20, p. 38–61.

(15) Temple, J. C. 1911 The influence of stall manure upon the bacterial flora of the soil. Ga. Agr. Exp. Sta. Bul. 95.

(16) Waksman, S. A. 1922 Microbiological analysis of soil as an index of soil fertility. I. Mathematical interpretation of results obtained from a bacteriological analysis of soils. *In* Soil Sci., v. 14, p. 81–101.

(17) Waksman, S. A. 1922 Microbiological analysis of soil as an index of soil fertility. II. Methods of study of numbers of microörganisms in the soil. *In* Soil Sci., v. 14, p. 283–298.

(18) Waksman, S. A. 1922 The influence of reaction upon the growth of actinomycetes *In* Soil Sci., v. 14, p. 61–80.

SILICA AND SILICATES IN RELATION TO PLANT GROWTH AND COMPOSITION

C. J. SCHOLLENBERGER

Ohio Agricultural Experiment Station

Received for publication January 17, 1922

INTRODUCTORY

The functions of silica in plant economy are but little understood. Most writers on plant physiology class silicon with the non-essential elements, but with the reservation that although it may be dispensed with, many plants do better when it is present, if for no other reason than that they are accustomed to it. Deposits of silica are supposed to serve a useful purpose by hardening the outer walls of the plant stems, sharpening the edges of leaf blades or stiffening hairs, all of which are protective agents.

It has been thought that silica serves to stiffen the stems of cereals, and that fertilization with soluble silicates would thus be useful in preventing lodging of the crop, but according to Sachs (13, p. 289) the theory has no basis in fact. Pfeffer (12, vol. 1, p. 414) suggests that silicon may function like calcium in helping further to reduce the amount of potassium or phosphorus necessary for development. Certain plots at Rothamsted are dressed with sodium silicate and on the phosphate-starved plots the silicate appears to have caused marked increases in the barley crop. Hall and Morison (7) have made a study of these plots and conclude that the silicate causes an increased assimilation of phosphorus, the seat of action being in the plant and not in the soil. That is, the influence of the silicate upon the plant has enabled it to secure an increased amount of phosphorus from the soil, although from the lack of phosphorus fertilization the soil had become highly deficient in that element. A connection between the presence of silica and utilization of phosphorus had been noted by Kreuzhage and Wolff (9) from the results of solution-culture experiments with oats. In their work, the silica had been dissolved in the minimum amount of potassium hydroxide and added to the solution acid with sufficient nitric acid to neutralize the alkali and retain the silica in colloidal solution. Jennings (8) grew wheat seedlings in various colloidal jellies, and found that 1-per cent silica jelly was of great benefit to growth, while an agar jelly was less beneficial and under some circumstances was distinctly harmful. He concludes that silica is beneficial because absorbed and actually used by the plant.

Cowles (4) and Scheidt (14) have published data showing beneficial results to various crops from applications of dicalcium silicate, a by-product from the manufacture of sodium aluminate. This material contains a readily decomposible calcium silicate, also a certain amount of water-soluble sodium silicate. They attribute its value to both the calcium and silica contained. Conner (3) reports that dicalcium silicate gave better results in field and pot experiments than were obtained from the use of lime. This is attributed to the superior effect of the active silicate in reducing the amount of aluminum in the soil solution, which Connor considers to be the most harmful factor in acid soils. At equivalent hydrogen-ion concentration, aluminum is less soluble as silicate than as hydrate, according to data reported in the article cited.

It has been shown in this laboratory that the bases of dicalcium silicate are quite as effective in reducing lime requirement of soil as are those of ground limestone under similar conditions. Ground blast furnace slag was included in this test, and while its activity was less than that of the other materials mentioned, it was quite appreciable (15). While the bases of slag have thus been shown to be less available, or less active in satisfying the soil's absorptive capacity for bases, it does not necessarily follow that the effect of slag upon plant growth will be inferior to that of limestone. It may possibly have virtues peculiar to itself, and only to be revealed by practical trial. Pot tests have indicated that both finely ground blast furnace slag and precipitated calcium silicate are superior to precipitated calcium carbonate used at the same rate, 4400 pounds per acre, when tested with red clover as the crop (1). And further, the value of several natural silicates as soil amendments in pot experiments has been demonstrated by MacIntire and Willis (10).

OBJECTS OF EXPERIMENT

Consideration of this earlier work led to the belief that instructive results might be obtained from pot experiments in which several carriers of silica and both soluble and insoluble phosphates were applied to various pots, and crops grown under conditions approaching normal. The amounts of silica taken up by plants from that offered in various forms, the effect of applications of calcium carbonate upon the assimilation of silica and the effect of silica upon the amounts of phosphorus utilized by plants from that applied to the soil as soluble monocalcium phosphate and as rock phosphate (floats) were other points considered in planning the experiment.

In particular, it was desired to determine whether the demonstrated value of blast furnace slag is due partly to its silica content, or whether the bases supplied are the sole cause of its beneficial effects. The great quantities of blast furnace slag available in many localities having soils in need of liming, and the apparent effectiveness of the material as a substitute for ground limestone as a soil amendment, led to its inclusion in the present investigation. Furthermore, the possibility suggested itself that blast furnace slag might be both a sufficient source of bases for acid soils and of silica for plants, and that the silica thus supplied might be of positive benefit to crops, especially with respect to increasing the possible utilization of insoluble phosphate, as the work previously cited has suggested. There seems to be still another reason why the use of slag instead of limestone with rock phosphate might be advantageous. Both theory and results of practical field experiments indicate that rock phosphate is most effective when used on a soil not too abundantly supplied with bases. Yet the maintenance of fertility is not possible without legumes, which require lime in some form. A material like slag, less active than the common forms of lime when finely divided and thoroughly distributed in the soil, might be able to supply sufficient calcium and sweeten the soil to an extent sufficient for a satisfactory growth of legumes without making soil conditions too unfavorable for the utilization of rock phosphate.

SOIL, MATERIALS AND PROCEDURE

The soil used for the experiment was Wooster silt loam obtained from the edge of one of the fertility fields on the Station farm; it is not known ever to have been limed, contains but a trace of carbonate and is acid to litmus paper and the Truog test. The soil to the depth of 6 to 7 inches and the subsoil down to 1 foot were taken up separately and after thorough mixing by shoveling were packed in their proper order in 20-inch sewer pipes sunk in the ground for pots. The subsoil was tamped in very firmly to make good contact with the native subsoil at the bottom. The top of the subsoil was within 7 inches from the top of pot. The pots were completely filled with thoroughly compacted soil and finally finished with a slightly convex surface. The object was to secure a very compact subsoil, as exists in the field, and to prevent abnormal conditions as a result of the soil shrinking away from the walls of the pot after a time. Calcium carbonate, silicate, hydrated silica, blast furnace slag and rock phosphate (floats) were applied by removing the upper 6 inches of soil, mixing the materials with it and replacing it in the same manner as it was first put in the pots. Sodium silicate (water glass) and dialyzed silica were diluted with water, sprinkled over the soil removed as stated and the whole mixed. After allowing the mixture to dry, lumps were crushed and the soil was again mixed and replaced in the pot. Potassium and nitrogen were added to all pots in solution containing 70 cc. of 10-per cent potassium nitrate and soluble phosphorus by 73 cc. of 10-per cent monocalcium phosphate where indicated. One or both portions of the solution were diluted to 250 cc. and distributed over the surface of the pots as evenly as possible by means of a separatory funnel. After drying, the soil was thoroughly mixed and put into optimum condition for seeding. Pots receiving soluble phosphate had the equivalent of 2600 pounds per acre of fertilizer containing 2 per cent ammonia, 7 per cent phosphoric acid and 5½ per cent potash. All received nitrogen and potash at the same rate. Rock phosphate was applied at the rate of 1100 pounds per acre; the commercial ground rock was used.

The calcium carbonate used was Baker's Analyzed; each pot limed received 182 gm. which is equivalent to 4 tons per acre. The calcium silicate, corresponding to the formula $CaSiO_3$, was prepared by the interaction of solutions of calcium chloride and sodium silicate. This and blast furnace slag were used at the rate of 100 gm. per pot. The hydrated silica was produced by adding excess of sulfuric acid to diluted water glass, evaporating and washing; pots receiving this also had 200 cc. dialyzed silica, made by adding diluted water glass to excess hydrochloric acid, and dialyzing in collodion bags until nearly free from chlorine. It was still liquid when applied but could be caused to set to a jelly. The blast furnace slag was ground to pass a 100-mesh sieve, and was from the same lot as that employed in other tests previously reported (1, 15). All treatments with silicon compounds were intended to furnish approximately 1000 pounds per acre silicon, and the quantities applied were dependent upon the composition of the material.

Crops on these pots were treated so far as possible the same as those in the field with respect to time of seeding and were found to ripen at practically the same time.

FACTORS AFFECTING YIELDS

The variously treated pots were 30 in number, and were situated in a wire cage with similar pots used for other experiments. The materials applied to individual pots are indicated in table 1, together with yields and composition of crops from pots so treated, and need not be enumerated here. There were sixteen variations in treatment, all but two of which were in duplicate pots. The slag-treated pots were all in one row with duplicates adjacent, but duplicates of other treatments were in separate rows, about 2 rods apart, with the order of treatments reversed. The object of this separation was to determine differences due to local factors, and to prevent any erroneous conclusions. Results obtained indicate that this is a point of some importance, as row 33 was remarkably superior in appearance and yield to the duplicate pots of row 15 in the case of the soybeans grown in 1920. Even in the cases of the adjacent duplicates in row 13, differences in yield and composition are considerable, although there is no consistency which would indicate that such differences were due to more than temporary influences. It is very difficult, if not indeed impossible, to avoid great variability in work of this nature. Even in solution cultures, with every precaution possible, it has been shown (5) that individual plants may vary in dry weight by 50 per cent and duplicate cultures of six plants each by 20 per cent from the mean of 33. While the influence of this factor of variability in individual plants should be less in the case of these pots, which had an average of 30 soybean plants in 1918 and about 25 in 1920, it must still exert an appreciable influence, although probably insignificant in comparison with that of unavoidable differences in preparation and subsequent treatment of pots. The pots were seeded quite thickly, with the idea of thinning, the plants later to secure a uniform stand; after thinning, however, a few plants were accidentally lost. This factor, however, does not appear to have had much influence on the total yield of a pot, as the largest difference between the pots of a pair in 1918 was five plants, a pot with 25 plants yielding 83 gm. crop, while its duplicate with 30 plants yielded but 66 gm. Another pot receiving nearly the same treatment and bearing 31 plants yielded 123 gm. Even those pots with the smallest number of plants had a heavier stand than is often seen in the field, and the plants came to maturity in a normal manner although greatly stunted on low yielding pots and always considerably smaller than plants in the field.

The first crop grown was soybeans; the season of 1918 was unfavorable, and the pots had to be watered with rather hard tap water. Either on account of the slight amount of calcium carbonate thus supplied, or because the soil was not as acid as had been thought, the unlimed pots bore quite as good a crop as those limed. Following the first crop of soybeans, it was decided to grow a cereal crop naturally high in silica, and the choice fell on oats. The treatment with fertilizers, i.e., nitrogen, potassium and soluble phosphorus only, was repeated. No additions of silica were made to any of the pots. The oats grown in 1919 suffered from the depredations of mice, which ate the seeds before they germinated, so that certain pots had to be replanted. Pots 9 and 10 in row 15, and 6 to 10 in row 33 were replanted, some a third time. Yields were doubtless affected by the later planting. The pots were watered with tap water to some extent, but not so much as in 1918. The difficulties encountered with the oats led to the choice of soybeans again for the third crop. A second application of all the various treatments was made at the same rates as when first used, with the exception of calcium carbonate, which was applied at half the former rate. The season of 1920 was exceptionally favorable for soybeans, as may be seen from the large yields of the pots. Rains were regular during the growing season, and the pots were not artificially watered at any time. For that reason, perhaps, or possibly on account of the very rapid depletion of calcium from soils in cylinder experiments, as noted by Mooers (11), the yields from the pots without additions of basic materials are very small. The plants in pot 9, row 13, shed their leaves prematurely, so that the yield recorded was mostly stems, a circumstance reflected in the high silica and low phosphorus content of the crop from this pot. There were no pods borne on these plants, but those on pot 10 of the same row had a few. Buckwheat was the last crop grown, in 1921. It made a satisfactory growth on all the pots except those without lime or slag. On these, the seed germinated in a normal manner, but the plants grew scarcely at all. When the other plants had matured seed and were harvested, only a few stunted plants were still alive on these pots. The weights of dried crop secured were too small even to furnish samples for analysis. The crop suffered somewhat from dry weather, but was not artificially watered. The plants bloomed profusely but seed production was quite light.

ANALYTICAL METHODS

Grain and straw were always ground together in preparing the samples for analysis. Total silica was determined by ashing 20 gm. of the finely ground sample in a platinum dish at the lowest possible temperature, finishing in an electric muffle furnace held at 500–550°C. The dishes were allowed to cool, covered, water and hydrochloric acid added and evaporated to dryness, finally baking at 120 to 130° to dehydrate silica. The residue was moistened with strong hydrochloric acid, diluted and digested until salts were in solution, and filtered on a retentive paper. The thoroughly washed silica was ignited in platinum, weighed, evaporated with 15 cc. hydrofluoric acid plus a few drops sulfuric acid, ignited and weighed again. Total silica is thus obtained by difference. Sand was determined in a similar manner, but instead of the separated silica being ignited, it was washed from the filter back into the dish, evaporated, 20–40 cc. 5 *N* sodium carbonate solution added and boiled about 5 minutes, being kept covered while boiling. Some hot water and a few drops of strong sodium hydroxide solution were added, again brought to boiling, allowed to settle and carefully decanted onto the same filter. The residue in the dish was treated twice more with 20 cc. portions of the sodium carbonate, and was finally washed with water, then with dilute acid and finally thoroughly with hot water. The silica in this residue of "sand" was determined as previously described, and subtracted from total silica gives *plant silica.* In order to check the work, the soluble silica in the alkaline filtrates was determined directly in numerous cases. Good agreement was always obtained. The procedure described is the same in principle as the official method (2, p. 15); a slight modification was designed to shorten the method. Dilution after boiling with sodium carbonate solution is not mentioned in the official method, but was found essential with samples high in silica; if not done, the dissolved silica is likely to gelatinize on the filter, and thus cause much extra work if the determination is to be saved.

The determination of "plant silica" is not by any means perfect. The data obtained from the soybeans grown in 1918 gave rise to the suspicion that silica in sand had been appreciably attacked and was included with the plant silica, since the percentages of silica in sand were much higher than those obtained from the soybeans grown in 1920, while plant silica percentages were considerably higher and showed a tendency to vary with the sand. It should be noted that the sodium-carbonate-insoluble silica is not all supplied by sand. Under the microscope, sand grains were almost absent from some of the residues. The bulk of the material was very fine and from the nature of the residue left after treatment with hydrofluoric and sulfuric acids, it was judged to be of clayey nature. The fact that it was much less in 1920 is due to the frequent rains of that season, which both washed the leaves of the plants and reduced windblown soil to a minimum. It is possible to employ a correction formula, after the plan of Dyer (6), to allow for silica dissolved from sand, and to do so seems to improve the data. One such formula, founded on the assumption that only 75 per cent of the extraneous silica was obtained as sand, appeared to fit the data obtained from both the 1918 and 1920 crops of soybeans very well and made the results quite concordant. The percentage of plant silica in the two crops of soybeans contained in table 1 are calculated on this basis, i.e., the percentages of plant silica as indicated by the analytical data are *decreased* by one-third the amount of silica insoluble in sodium carbonate solution. The same procedure applied to the data obtained from analyses of oats, however, causes no improvement, nor indeed is it to be expected that the same formula could apply in both cases. The oats plant is much higher in total and sodium carbonate soluble silica, and the consequent difficulty in dissolving all the plant silica would tend toward low results, rather than high for sodium carbonate soluble silica. Furthermore, the ash of oats plants contains practically no carbonate, while soybean-plant ash is high in carbonate, a factor which can hardly be without influence on the amount of extraneous silica attacked during the ignition, in spite of all precautions to keep the temperature low. For these reasons, it is thought that analytical errors due to attack of sand and resistance of plant silica are more nearly balanced in the data for oats, and the percentages of plant silica in the crop tabulated are those actually obtained. The buckwheat is so low in silica that no effort at correction seems advisable. In all cases, the percentages of "sand silica" tabulated are those actually found by analysis.

Total phosphorus was determined by the official method (2, p. 18) and weighed as magnesium pyrophosphate.

DISCUSSION OF DATA

The yields in grams of air-dry crop (all parts above ground) and percentages of silica insoluble in 5 *N* sodium carbonate solution (sand silica) and soluble in that reagent (plant silica) and total phosphorus in the crops grown in the pots, are presented in table 1. The only reason for tabulating the percentages of extraneous silica (sand) is the possible influence of variations in this constituent upon the figures obtained for plant silica, as has been explained. To facilitate discussion of the effects of the different treatments, each is designated by a letter in the table.

The effect of treatment with blast furnace slag will be first discussed. A comparison of data from treatments A, B and G, rock phosphate (floats) and blast furnace slag with and without calcium carbonate, and phosphate and carbonate together, does not indicate that slag is inferior to precipitated carbonate with respect to weights of crop produced. The slag has supplied sufficient base. Data for plant silica in both crops of soybeans indicate that the slag has supplied a considerable amount of silica, which has been assimilated by the soybeans. Variation between duplicate pots is too great with the oats, and there is no suggestion of a similar effect with the buckwheat. On the other hand, buckwheat is the only crop furnishing data which suggest that the slag has been of any aid in the utilization of the phosphorus of rock phosphate. This effect seems apparent only in the absence of calcium carbonate, and unfortunately the crop failed on the pot (E) without slag or carbonate, so that there is no indication of how much phosphorus the plant might have taken up from the raw phosphate in the absence of both basic materials.

The treatments C, D and L are identical with those just discussed, aside from the substitution of the soluble monocalcium phosphate for the rock phosphate. With one or two exceptions, the largest yields were obtained from the treatment C with both slag and calcium carbonate. The availability of the silica in slag is again indicated, but calcium carbonate has reduced the amount assimilated. All crops after the first have given decisive indications of the ready availability of the soluble phosphate supplied, but there is no evidence that the silica in slag had any effect upon this.

The yield of soybeans from the first crop grown in 1918, on the pots receiving the treatments which have been discussed and considering treatments E and F

TABLE 1

Yields, silica in "sand," "plant silica" and phosphorus in crops

ROW NUMBER	POT NUMBER	TREATMENT	SOYBEANS, 1918				SOYBEANS, 1920				OATS, 1919				BUCKWHEAT, 1921			
			Crop	Silica		Phosphorus	Crop	Silica		Phosphorus	Crop	Silica		Phosphorus	Crop	Silica		Phosphorus
				Sand	Plant			Sand	Plant			Sand	Plant			Sand	Plant	
			gm.	*per cent*	*per cent*	*per cent*	*gm.*	*per cent*	*per cent*	*per cent*	*gm.*	*per cent*	*per cent*	*per cent*	*gm.*	*per cent*	*per cent*	*per cent*
13	1	A: Floats, blast furnace slag, $CaCO_3$.	83	0.34	0.25	0.17	231	0.16	0.21	0.14	106	0.69	1.79	0.24	74	0.18	0.12	0.16
13	2		66	0.60	0.18	0.11	269	0.11	0.23	0.17	102	0.44	1.32	0.21	74	0.20	0.12	0.14
13	3	B: Floats, blast furnace slag.	78	0.44	0.32	0.12	241	0.13	0.24	0.19	103	0.41	1.59	0.22	59	0.22	0.11	0.19
13	4		80	0.57	0.31	0.12	217	0.12	0.24	0.19	108	0.54	1.65	0.23	72	0.20	0.13	0.18
13	5	C: Soluble phosphate, blast furnace slag, $CaCO_3$.	90	0.57	0.20	0.15	313	0.13	0.24	0.25	126	0.52	1.31	0.26	84	0.15	0.09	0.30
13	6		104	0.44	0.16	0.17	370	0.13	0.20	0.25	135	0.48	1.35	0.28	84	0.13	0.09	0.29
13	7	D: Soluble phosphate, blast furnace slag.	75	0.58	0.23	0.12	321	0.13	0.26	0.25	98	0.62	1.74	0.25	75	0.16	0.11	0.31
13	8		90	0.52	0.30	0.14	313	0.11	0.26	0.26	63	1.40	1.60	0.31	82	0.21	0.12	0.28
13	9	E: Floats.	85	0.54	0.26	0.19	18	0.65	0.23	0.19	56	1.07	1.40	0.32	4			
13	10	F: Soluble phosphate.	92	0.63	0.24	0.16	31	0.27	0.18	0.26	83	0.40	1.37	0.23	5			
15	1	G: Floats, $CaCO_3$.	95	0.64	0.10	0.18	178	0.15	0.11	0.21	79	0.45	1.20	0.24	76	0.26	0.14	0.15
33	10		77	0.49	0.11	0.16	313	0.15	0.15	0.17	56	0.70	1.71	0.33	60	0.21	0.12	0.15
15	2	H: Floats, $CaSiO_3$, $CaCO_3$.	123	0.55	0.16	0.21	178	0.18	0.21	0.19	102	0.85	1.62	0.29	73	0.21	0.09	0.16
33	9		82	0.43	0.25	0.16	311	0.14	0.26	0.18	79	0.57	2.19	0.30	75	0.19	0.14	0.17

15	3	I: Soluble phosphate hydrated silica, $CaCO_3$.	102	0.46	0.31	0.18	238	0.14	0.28	0.24	109	0.49	1.79	0.25	73	0.23	0.09	0.29
33	8		112	0.39	0.23	0.18	177	0.19	0.27	0.24	87	0.72	1.72	0.30	85	0.26	0.15	0.30
15	4	J: Soluble phosphate, $CaSiO_3$, $CaCO_3$.	101	0.72	0.30	0.19	284	0.13	0.26	0.20	87	0.74	1.83	0.33	80	0.52	0.16	0.28
33	7		131	0.46	0.23	0.15	251	0.68	0.31	0.22	99	0.45	2.03	0.28	85	0.23	0.12	0.31
15	5	K: Soluble phosphate, water glass, $CaCO_3$.	85	0.64	0.33	0.18	303	0.36	0.29	0.27	99	0.38	1.80	0.29	81	0.18	0.08	0.35
33	6		123	0.32	0.24	0.20	244	0.18	0.34	0.25	93	0.35	1.85	0.29	68	0.27	0.10	0.29
15	6	L: Soluble phosphate, $CaCO_3$.	97	0.59	0.18	0.16	258	0.14	0.15	0.24	74	0.73	1.07	0.30	72	0.29	0.06	0.35
33	5		110	0.45	0.16	0.16	312	0.18	0.12	0.24	88	0.50	1.30	0.31	73	0.27	0.09	0.25
15	7	M: Hydrated silica, $CaCO_3$.	96	0.73	0.25	0.17	230	0.14	0.26	0.20	70	1.26	1.90	0.31	31	0.33	0.12	0.09
33	4		99	0.58	0.21	0.19	279	0.15	0.20	0.20	81	0.53	1.83	0.30	53	0.25	0.09	0.12
15	8	N: $CaSiO_3$, $CaCO_3$.	88	0.78	0.18	0.16	218	0.14	0.27	0.15	96	0.91	1.60	0.26	50	0.20	0.08	0.11
33	3		106	0.58	0.20	0.21	300	0.15	0.23	0.21	95	0.54	1.63	0.28	76	0.22	0.10	0.15
15	9	O: Water glass, $CaCO_3$.	67	0.66	0.27	0.13	178	0.14	0.35	0.20	88	0.54	1.86	0.26	51	0.40	0.21	0.31
33	2		104	0.38	0.21	0.19	244	0.18	0.30	0.23	134	0.38	1.72	0.23	79	0.22	0.13	0.16
15	10	P: $CaCO_3$.	77	0.79	0.08	0.14	171	0.16	0.15	0.15	50	0.60	1.26	0.24	35	0.29	0.11	0.09
33	1		79	0.54	0.14	0.18	286	0.15	0.13	0.18	99	0.43	1.42	0.26	41	0.23	0.10	0.12

which had no additions of basic material, give some reason for the belief that the slag may have had a slightly unfavorable influence the first season. This is not apparent in later crops, although a second full application of slag and half application of calcium carbonate were made preceding the soybeans grown in 1920.

The effect of the lack of basic materials in treatments E and F began to be noticeable at the second crop, and caused a practical failure at the fourth crop. Pots receiving slag as the only basic material compare well with those receiving calcium carbonate, although the latter was applied in larger amount. Data obtained from this experiment indicate that slag is an effective substitute for calcium carbonate; there is little evidence that it has a favorable effect in any other respect.

The treatments G and H, rock phosphate and calcium carbonate alone and with the addition of calcium silicate were intended to indicate whether a silicate presumably more active than slag would have a similar effect when used in connection with rock phosphate. Yields of the first and second crops appear to have been increased by the calcium silicate. The plant silica content of all the crops excepting buckwheat appears to have been appreciably increased by this substance. The phosphorus content of crops has been slightly increased in several instances where the silicate was used.

The treatments J and L are similar, save that the phosphorus was in a soluble form. Again the yields are slightly increased by calcium silicate in the greater number of instances; plant silica is considerably increased in all cases, but the effect on phosphorus is not plainly indicated.

In the treatments N and P, calcium silicate and carbonate and carbonate alone, we have another opportunity to note the effect of calcium silicate, this time when used without any phosphorus carrier. The averaged yield of the pair of pots on which the silicate was used is larger than the yield where carbonate only was applied. The plant silica content is higher except in the case of buckwheat, and the averaged percentages of phosphorus are slightly higher.

Sodium silicate (water glass) was the source of silica for two treatments, K with soluble phosphate and calcium carbonate, and O with calcium carbonate only in addition to the sodium silicate. Comparing these with the pair of pots P, which received calcium carbonate only, it is seen that K has usually borne the largest crops, as would be expected from the fact that this treatment included available phosphorus. The crop from these pots has likewise been highest in phosphorus. The silica of water glass has been absorbed to a considerable extent by both soybeans and oats, apparently but little by buckwheat, unless the figure for plant silica in the crop from pot 9 in row 15 is accepted. The determinations on the sample from both this pot and its duplicate were repeated, but the same results were obtained. The effect of the water glass upon the phosphorus content of the crop from the pots without phosphorus treatment is not definitely indicated. Soybeans of 1918 indicated no effect, as did oats in 1919, save that the crop was larger where water glass was applied. Soybeans of 1920 indicated a marked increase in phosphorus content, and the buckwheat a remarkable increase, but the high figure is again dependent upon a sample which gave abnormal results for another constituent.

The treatments with silicates discussed have been complicated somewhat by the extra base introduced by the silica carrier. The treatments I and M were designed to show the effect of silica itself. Hydrated silica, prepared by acidifying and evaporating water glass and washing the precipitate, and dialyzed silica in addition, were applied to these pots, together with calcium carbonate, and in one case, soluble phosphate. Except with soybeans in 1920, treatment I, including phosphate, has yielded most crop. In the other years, the hydrated silica has shown practically no effect upon yields as compared with the effect of calcium carbonate alone. The silica offered in this form has been assimilated, as is shown by a comparison between the composition of crops from pots so treated and from those pots having treatment P, calcium carbonate without added silica. An inspection of the data for phosphorus content of the crops from the pots discussed would seem to indicate that the added silica has resulted in a slight increase in phosphorus content of every crop except buckwheat.

SUPPLEMENTARY TEST FOR COMPARISON OF SILICATES WITH CARBONATES

This was started in 1914, and conducted in 20-inch sewer pipe cylinders in a manner similar to the test which has been described. The comparison included sulfur as a part of the treatment on several pots, as it was thought that the sulfur content of slag might be responsible for some of its beneficial action. The pots treated with powdered sulfur at the rate of 5 gm. each will not be considered, as the yields fell off very rapidly, evidently on account of the increasing acidity. Pot 10 received two much smaller applications of sulfur the first and second years of the test, none thereafter, but was fertilized the third season. The soil was of the same type as that employed for the experiment previously described, but was taken from a slope long uncultivated and was found to have an appreciably greater reserve supply of bases. The blast furnace slag and other materials were from the same lots as those used in the other experiment described; the slag was ground to pass a 100-mesh sieve. In this case, slag, silicate and carbonate were applied at the same rate, 100 gm. per pot, equivalent to 4400 pounds per acre. The fertilized pots received 3 gm. dried blood, 1.5 gm. potassium chloride and 1.7 gm. calcium phosphate each. The phosphate used was the dibasic salt.

Yields and, in some cases, phosphorus contents of crops from these pots are presented in table 2. The first crop of red clover gave the largest yield on the pots treated with calcium silicate, but the second cutting was largest on the slag treated pots. The total produced for the season averaged nearly the same on each pair of pots, fertilized and unfertilized together.

TABLE 2

Air-dry weights and phosphorus content of crops in supplementary test, Row 19

POT	TREATMENT	RED CLOVER, 1914–1915		WHEAT, 1915–1916						SOYBEANS, 1917		SOYBEANS, 1918	OATS, 1919	SOYBEANS, 1920	BUCK-WHEAT, 1921		TOTAL CROP, SEVEN YEARS
				Grain		Straw		Plant									
		First cutting	Second cutting	Crop	Phosphorus	Crop	Phosphorus	Crop	Phosphorus	Crop	Phosphorus	Crop	Crop	Crop	Crop	Phosphorus	
		gm.	*gm.*	*gm.*	*per cent*	*gm.*	*per cent*	*gm.*	*per cent*	*gm.*	*per cent*	*gm.*	*gm.*	*gm.*	*gm.*	*per cent*	*gm.*
1	Check	137	70	55	0.43	96	0.09	151	0.22	93		66	49	71	10		647
10	Sulfur, fertilizer later	158	50	69	0.42	129	0.09	198	0.20	65		48	59	39	3		620
2	100 gm. slag	181	72	80	0.41	140	0.07	220	0.20	155	0.18	137	69	185	47	0.15	1066
3	Same with fertilizer	195	89	75	0.42	122	0.07	197	0.20	148	0.22	140	81	185	86		1121
4	100 gm. $CaSiO_3$	204	90	102	0.41	200	0.08	302	0.19	134	0.20	115	98	144	35	0.18	1122
5	Same with fertilizer	195	52	81	0.42	142	0.07	223	0.19	145	0.20	110	86	184	15		1010
8	100 gm. $CaCO_3$	148	62	68	0.43	119	0.12	187	0.23	130	0.17	104	61	270	37	0.15	999
9	Same with fertilizer	142	42	52	0.44	86	0.11	138	0.24	102	0.17	94	42	308	22		890

Calcium silicate seems to have been of most benefit to the wheat harvested in 1916; the phosphorus contents of the wheat are found to have an inverse relation to the yields, which are lowest on the pair of pots treated with calcium carbonate, but the crops grown on these pots carry the highest percentage of phosphorus.

Previous to planting the 1917 crop of soybeans, the pots were given another application of basic materials at the same rate as at the beginning. The formula for the application of fertilizer was changed, thus: dried blood, 2.5 gm.; sodium nitrate, 0.5 gm.; dibasic calcium phosphate, 2.7 gm., and potaspotassium chloride, 1.2 gm., were applied dry to each pot. Slag appeared to be of greatest benefit to soybeans, with calcium silicate next best. The connection between yields and phosphorus content of the crop noted in the case of the wheat was not apparent in the soybean crop. The indications of the soybeans grown in 1918 were practically the same as given by the crop of the previous season; yields were less, however.

The slight increases or, quite commonly, marked decreases in the crop attributable to fertilization of these pots caused the abandonment of this part of the program. After 1917, no fertilizer was applied. The oats crop of 1919 did best on the pots treated with calcium silicate; the slag treated pots were next best. The soybeans grown in the exceptionally favorable year of 1920 gave a surprising yield on the pots receiving calcium carbonate, far better than that obtained from the pots receiving silicates, which yielded about the same. The next crop, buckwheat grown in 1921, saw the slag treated pots again the best yielders, but calcium carbonate was second. The check pots, which had been gradually declining in yield, were nearly complete failures in 1921. Number 10, which had received sulfur the first two seasons, made the poorest showing. The total weight of crop produced in 7 years is nearly the same for blast furnace slag and calcium silicate. Calcium carbonate was apparently more beneficial in the later years of the test than it had been at first, but in total weight of crops the two pots treated with carbonate are distinctly inferior to those receiving the silicate and blast furnace slag.

RESULTS OF FIELD TEST OF SLAG AND LIMESTONE

The agronomy department at this Station has conducted an investigation of the influence of various factors upon the physical characteristics of the wheat plant. Included in this work are two small plots, one dressed with slag at the rate of 6000 pounds per acre and the other with commercial ground limestone at half the above rate. In addition to the disparity in size of application, the factor of fineness of material also was probably in favor of the slag, as it was ground to pass a 20-mesh sieve, and 56 per cent of the material passed a sieve having 100 meshes per linear inch. The mechanical analysis of the limestone is not definitely known, but it was certainly coarser than the slag, since screen separation of similar lots of stone from the same source showed that 95 per cent or more passed 10 mesh and only 30 to 40 per cent passed 100 mesh.

Small samples of mature wheat plants were taken from these plots, dried and after determining the amount and characteristics of the grain, the whole sample of grain and straw together was ground for analysis. The results of the analytical work are presented in table 3, together with acre yields based on single plots 10 feet square. The yields recorded would indicate that the slag had been beneficial. No explanation is offered for the low yield on the plot receiving limestone. The percentage relation of grain to the whole plant above ground is lowest in the case of the slag treated plot. The weights of 50 grains tabulated indicate that grain from this same plot is least well developed. The low percentage of phosphorus in the plants from this slag treated plot is doubtless connected with the smaller proportion of grain in the whole plants, and might lead to the belief that a part of the grain had been lost, but the fact that the grain from this plot is lightest does not support this explanation.

TABLE 3

Yields and composition of wheat plants from slag, limestone, and check plots

TREATMENT	YIELD PER ACRE	GRAIN IN SMALL SAMPLES	WEIGHT OF 50 GRAINS	SILICA		PHOSPHORUS
				In sand	In plant	
	bus.	*per cent*	*gm.*	*per cent*	*per cent*	*per cent*
Check	18.1	25.5	1.27	0.30	2.11	0.213
Slag	21.1	19 8	1.14	0.35	2.80	0.176
Limestone	12.6	23.0	1.17	0.32	2.32	0.212

On the whole, it does not seem unreasonable to suppose that the large application of finely ground slag to the soil has resulted in an excess of nitrogen being made available. This would result in a greater vegetative growth, both by stooling and by the formation of straw, with but a slightly larger total yield of grain not well filled out. The wheat plants from the plot treated with slag are highest in plant silica, but the data do not indicate that this silicate has aided in the assimilation of phosphorus and thus tended toward improvement in quality of the grain.

SUMMARY

In this paper are reported the results of two series of pot experiments and one field experiment on small plots, designed to determine the effect of blast furnace slag and other silica carriers with reference to supply of bases to the soil and aid in assimilation of phosphorus. Data from which conclusions have been drawn have included yields and chemical composition of the plants grown.

One series of pot experiments included thirty 20-inch cylinders with sixteen treatments, and was designed to show the effect of silica applied in various forms to an acid silt loam soil with and without calcium carbonate, rock phosphate or soluble calcium phosphate. Data from two crops of soybeans and one each of oats and buckwheat are available for drawing conclusions, which may be summarized as follows: Silica, added as air-dry hydrated silica plus dialyzed silica, blast furnace slag, calcium silicate and sodium silicate, was assimilated, as shown by increased percentages of plant silica in the crops. The silica was generally increasingly available from the compounds in the order given. Buckwheat showed smallest differences in silica content as the result of treatment, oats the greatest. Additions of calcium carbonate usually appeared to lessen the amounts of silica taken up by the plants.

Ground blast furnace slag is an effective source of bases and its silica is to some extent assimilated, but conclusive evidence that the silica thus added to the plant is of use in enabling the plant to utilize more phosphorus from rock phosphate was not obtained. Except for buckwheat, calcium silicate is also an effective source of silica. The silica from this source appeared to enable most of the crops to obtain more phosphorus, when that element was added as rock phosphate.

Pots treated with various silica compounds and soluble phosphates furnished no data on which to base conclusions as to the action of silica in phosphorus assimilation. A majority of the pots to which additions of silica compounds with calcium carbonate were made showed increased yields and phosphorus content in the crop as compared with pots receiving calcium carbonate only, the soil being the sole source of phosphorus.

A second series of eight cylinders, similar to those described, furnish a comparison of the effects of equal weights of slag, precipitated calcium silicate and carbonate, with and without a complete fertilizer. Seven crops were grown on this series of pots and with the exception of one season, yields on the pots to which the silicates were applied were greater than yields from check pots or those receiving carbonate.

Benefit from calcium carbonate was unmistakable only after the second year, but became increasingly noticeable with time. Of the three crops from which samples were analyzed, only the soybeans showed any consistent relation between silica and phosphorus utilization. With this crop, the plants were higher in phosphorus on those pots where silicates were used instead of carbonates. The wheat crop indicated that the opposite was the case, but low yields accompanied the high phosphorus percentages.

A field test in which finely ground slag applied at the rate of 3 tons per acre was compared with half as much limestone, probably somewhat coarser, on wheat indicated that the slag caused a slightly larger yield of less well developed grain. This is thought to be due to a more abundant supply of available nitrogen on the plot to which the slag was applied. The slag caused a considerable increase in the silica content of the wheat plant, but phosphorus was decreased.

REFERENCES

(1) AMES, J. W. 1916 Blast furnace slag as a source of bases for acid soils. *In* Ohio Agr. Exp. Sta. Mon. Bul., v. 1, no. 12, p. 359–362.

(2) ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS 1919 Official and Tentative Methods of Analysis. Williams & Wilkins, Baltimore.

(3) CONNER, S. D. 1921 Liming in its relation to injurious inorganic compounds in the soil. *In* Jour. Amer. Soc. Agron., v. 13, no. 3, p. 113–124.

(4) COWLES, A. H. 1917 A new fertilizer containing calcium silicates and their value in supplying lime and soluble silica for improving plant growth; and why old experiments using soluble alkali silicates gave unfavorable results. *Presented at* Amer. Chem. Soc., Boston; *pamphlet by* The Electric Smelting and Aluminum Co., Sewaren, N. J.

(5) DAVIS, A. R. 1921 The variability of plants grown in water cultures. *In* Soil Sci., v. 7, p. 1–32.

(6) DYER, B. 1919 Estimation of silica and sand. *In* Analyst, v. 44, p. 28.

(7) HALL, A. D., and MORISON, C. G. T. 1906 On the function of silica in the nutrition of cereals—Part 1. *In* Proc. Royal Soc., series B, v. 77, p. 455–477.

(8) JENNINGS, D. S. 1919 The effect of certain colloidal substances on the growth of wheat seedlings. *In* Soil Sci., v. 7, p. 201–215.

(9) KREUZHAGE, C., and WOLFF, E. 1884 Bedeutung der Kieselsaüre für die Entwickelung der Haferpflanze nach Versuchen in Wassercultur. *In* Landw. Vers. Stat., v. 30, p. 161–197.

(10) MACINTIRE, W. H., and WILLIS, L. G. 1914 Comparison of silicates and carbonates as sources of lime and magnesia for plants. *In* Jour. Indus. Engin. Chem., v. 6, no. 12, p. 1005–1008.

(11) MOOERS, C. A. 1919 Abnormality of soils in field-placed cylinder experiments. *In* Soil Sci., v. 7, no. 4, p. 247–251.

(12) PFEFFER, W. 1900 The Physiology of Plants, v. 1, p. 632. *Trans. by* Alfred J. Ewart. Clarendon Press, Oxford.

(13) SACHS, J. VON. 1887 Lectures on the Physiology of Plants, p. 836. *Trans. by* H. Marshall Ward. Clarendon Press, Oxford.

(14) SCHEIDT, A. W. 1917 A comparison of calcium silicate, carbonate and hydrate as fertilizer materials with experimental data. *Presented at* Amer. Chem Soc., Boston; *pamphlet by* The Electric Smelting and Aluminum Co., Sewaren, N. J.

(15) SCHOLLENBERGER, C. J. 1921 Lime requirement and reaction of lime materials with soils. *In* Soil Sci., v. 11, p. 261–276.

THE SULFUR CONTENT OF RAINWATER

L. W. ERDMAN[1]

Iowa Agricultural Experiment Station

Received for publication January 25, 1922

Improved methods of sulfur analyses for soils and crops have revealed the necessity of studying the rôle which sulfur fertilizers may play in any system of permanent soil fertility. That sulfur is added to the soil in the rain and snow was recognized by early investigators, but the agricultural importance of this means of sulfur supply will depend largely upon the amount of sulfur found in rainwater falling in rural communities. Unfortunately most of the data relating to this subject have been obtained from analyses of rain falling in towns and cities.

Perhaps the earliest work on the analyses of rain for sulfur content is reported by Smith and summarized by Lawes, Gilbert and Warrington (10) as follows:

LOCALITY	H_2SO_4	POUNDS PER ACRE*	
		SO_3	S
	p.p.m.		
England, country inland	5.52	34.54	13.82
England, towns	34.27	214.70	85.88
Scotland, country seacoast	5.64	35.31	14.12
Scotland, country inland	2.06	12.89	5.16
Scotland, towns	16.50	103.32	41.33
Scotland, Glascow	70.19	439.77	175.91

* These figures were calculated on the basis of the data furnished by Lawes, Gilbert and Warrington that 2.41 p.p.m. of water amounted to 18.5 pounds per acre of SO_3.

Lawes, Gilbert and Warrington (11) found that the sulfuric acid (SO_3) contained in the rainwater at Rothamsted averaged for two years (1881–83) 2.41 parts per million of water amounting to 18.5 pounds per acre per annum. Hall (5) and Miller (13) made a summary of results from 1881–87 on the analyses of rainwater at Rothamsted and showed that the total amount of sulfur (SO_3) added yearly per acre was 17.41 pounds. Miller also reported that Gray found a yearly average of 14.94 pounds of sulfur (SO_3) per acre in the rainwater at Lincoln, New Zealand, and that Sestini found 20.89 pounds of sulfur (SO_3) in the precipitation at Catania, Sicily.

Crowther and Ruston (3) made a very extensive study of the rainwater falling at eleven different stations in the city of Leeds, England. The amounts of sulfur (SO_3) per acre varied from 91 pounds in the suburban residential section to as much as 336 pounds in the industrial area. At Garforth, six miles east of Leeds, they found an average annual precipitation of sulfur equivalent to 95.7 pounds SO_3 per acre. Later Crowther and Stewart

---

[1] The writer wishes to express his thanks to Dr. P. E. Brown for reading the manuscript.

(4) made further studies on the sulfur content of rain and reported results for 1912–1913 at Garforth to be 60 pounds, while in the polluted area of Leeds 352 pounds of sulfur (SO_3) per acre were obtained.

Wituynj (19) estimated the amount of sulfur in monthly samples of rainwater collected at eight different places in Russia from April, 1909–March, 1910. The results varied from 5.13 to 70.88 pounds of sulfur (SO_3) per acre, the larger amounts being obtained in the rain from the cities of St. Petersburg, Ochta and Mariupol. Kossovich (9) concluded from his studies that data for European Russia and available data for other countries indicated that the sulfur content varied between 8.92 pounds in the country and 72 pounds per acre per annum in the neighborhood of towns and industrial work, where the greatest portion falls in the winter.

Hart and Peterson (6) analyzed the rain falling at Madison, Wisconsin, from June, 1910, to October, 1910, and found the total amount of sulfur (SO_3) brought to an acre in five months was 10.7 pounds. They estimated a yearly fall of not less than 17 or 18 pounds.

Numerous analyses of rain and snow have been made at Mt. Vernon, Iowa, which may be summarized as follows:

INVESTIGATOR	PERIOD	RAINFALL	SO_3 PER ACRE
		inches	*lbs.*
Knox, W. K. (8)	Apr. 28, 1912–June 4, 1912	7.45	0.129
Artis, B. (1)	Oct. 12, 1914–June 9, 1915	18.49	4.913
Peck, E. L. (14)	Oct. 20, 1916–June 8, 1917	17.69	8.435
Trieschmann, J. E. (17)	Oct. 1, 1918–June 15, 1919	22.25	1.509
Shaffer, S. (15)	Aug. 18, 1920–June 1, 1921	20.97	327.0619

These data show that there is a wide variation in sulfur content of rain and snow from year to year. In the case of 1920–1921 the results are extremely high, and there is reason for belief that some discrepancy might exist in the calculations, because a comparison of the other substances reported by this investigator check fairly closely with the results of the previous investigators.

MacIntire, Willis and Holding (12) reported that the average analyses of three years' rainfall at Knoxville, Tennessee, showed an annual precipitation of 124 pounds of sulfur trioxide or 49.6 pounds of sulfur per acre.

Stewart (16, p. 107) reported as an average of seven years' work at Urbana, Illinois, that 45.1 pounds of sulfur was added to an acre annually and found that the amount of sulfur collected depended upon the amount of precipitation. Wilson (18) found that the rainwater collected at Ithaca, New York, supplied the soil with an average of 26.19 pounds of sulfur per acre annually.

The investigations reviewed show conclusively that the amount of sulfur reaching the soil through precipitation varies greatly with respect to the proximity of the sampling station to cities or towns. In order to study this means of the addition of sulfur to the soil under actual farm conditions, the writer placed a rain gauge on the agronomy farm of the Iowa Agricultural Experiment Station, which is two miles south of Ames. The results of the analyses of rain and snow for the year 1921 are reported in this paper.

Samples of rain and snow were collected in a 4-gallon glazed-stone crock placed close by a metal rain gauge of the U. S. Weather Bureau standard type. During the spring and summer months the crock was covered with two thicknesses of fine muslin between which was a large filter paper, to exclude particles of soil and other foreign material carried by the wind. The samples were stored in glass bottles and sealed with paraffined glass stoppers. Each month's samples were analyzed separately with the exceptions of January and February and November and December.

Sulfur in rainwater may occur chiefly as hydrogen sulfide, sulfur dioxide or sulfur trioxide. A method for total sulfur would necessarily require some oxidizing mixture to convert all the sulfur to the sulfate form. In this laboratory Benedict's (2) method for total sulfur in urine, with a few modifications, was found very accurate and convenient for the analysis of rainwater. Denis' sulfur reagent, as suggested by Hawk (7), was used instead of that employed by Benedict because it was easier to bring the solution to dryness during the last stage of evaporation. The method is as follows:

> Five cubic centimeters of Denis' reagent are added to 250 cc. of rainwater and portions of this solution are placed in an 80-cc. porcelain evaporating dish heated on an electric hot plate. When the 250 cc. have been evaporated to dryness the dish is placed in an electric furnace and heated to redness for 10 minutes after the black residue has become dry. After cooling, 10–20 cc. of dilute (1 :4) HCl are added to the residue in the dish which is warmed gradually until the contents have completely dissolved. The solution is filtered into a small Erlenmeyer flask and washed with cold distilled water to about 150 cc. The flask is placed on the electric hot plate, heated to boiling, and 10 cc. of 10 per cent $BaCl_2$ solution added drop by drop (5 cc. per minute), and allowed to stand overnight. The precipitate is filtered into weighed Gooch crucibles and washed free of chlorides with cold water (150 cc. in 15 cc. portions). The crucibles are dried at 110–120°C. for one hour and weighed. Controls should be run on the oxidizing mixture.

TABLE 1

Rainfall and content of sulfur for 1921

MONTH	RAINFALL	SULFUR CONTENT	
		SO_3	S
	inches	*lbs. per acre*	*lbs. per acre*
January and February	1.00	5.04	2.02
March	1.28	3.74	1.50
April	2.91	5.49	2.20
May	2.83	4.57	1.83
June	4.77	2.67	1.07
July	1.11	1.00	0.40
August	7.35	5.47	2.19
September	6.76	3.77	1.51
October	1.25	3.67	1.47
November and December	1.12	1.75	0.70
Totals	30.38	37.17	14.89

The results secured from analyses of rain and snow for total sulfur content from January 1 to December 31, 1921, are given in table 1. On account of the small precipitation it was necessary to combine the samples for January and February and also November with the December rain.

An examination of the data in the foregoing table shows for the year 1921 that there was a total rainfall of 30.38 inches, which is considered about normal for this section of Iowa. The sulfur content averaged for the year 3.32 parts per million parts of water. The total amount of sulfur brought to an acre of soil was 14.89 pounds, or an average monthly addition of 1.24 pounds per acre. With but few exceptions, the amounts of sulfur are fairly constant for each month, and there is no evidence whatever that the sulfur content depends directly upon the amount of precipitation.

It is believed that about 15 pounds of sulfur per acre per annum will represent a generally correct figure for rural communities. While this work reports only one year's data, the investigations at Rothamsted and other places show that the amount of sulfur added in the rainwater does not vary greatly from year to year.

The one conclusion to be drawn from this work is that under actual farm conditions the quantity of sulfur added to the soil per annum in the rainwater is not sufficient to overbalance the loss by drainage and cropping.

REFERENCES

(1) Artis, B. 1916 Nitrogen, chlorine and sulfates in rain and snow. *In* Chem. News, v. 113, p. 3–5.

(2) Benedict, S. R. 1909 The estimation of total sulfur in urine. *In* Jour. Biol. Chem., v. 6, p. 363.

(3) Crowther, C., and Ruston, A. G. 1911 The nature, distribution, and effects upon vegetation of atmospheric impurities in and near an industrial town. *In* Jour. Agr. Sci., v. 4, p. 25.

(4) Crowther, C., and Stewart, D. W. 1914 Further studies of the effects of smoke from towns upon vegetation in the surrounding areas. *In* Jour. Agr. Sci., v. 6, p. 396.

(5) Hall, A. D. 1905 The Book of Rothamsted Experiments, p. 21–22. E. P. Dutton & Co., London.

(6) Hart, E. B., and Peterson, W. H. 1911 Sulfur requirements of farm crops in relation to the soil and air supply. Wis. Agr. Exp. Sta. Res. Bul. 14.

(7) Hawk, P. B. 1916 Practical Physiological Chemistry, 5th ed., p. 548. P. Blakiston's Son & Co., Philadelphia.

(8) Knox, W. K. 1915 Nitrogen and chlorine in rain and snow. *In* Chem. News, v. 111, p. 61–62.

(9) Kossovich, P. S. 1913 On the circulation of sulfur and of chlorine on the earth, and on the importance of this process in the evolution of soil and in the plant world. *In* Zhur. Opytu. Agron. (Russ. Jour. Expt. Landw.), v. 14, p. 181–228; *abs. in* Exp. Sta. Rec., v. 30, p. 422.

(10) Lawes, J. B., Gilbert, J. H., and Warrington, R. 1881 On the amount and composition of the rain and drainage waters collected at Rothamsted. *In* Jour. Roy. Agr. Soc. Eng., v. 17, p. 266.

(11) Lawes, J. B., Gilbert, J. H., and Warrington, R. 1883 New determinations of ammonia, chlorine and sulfuric acid in the rainwater collected at Rothamsted. *In* Jour. Roy. Agr. Soc. Eng., v. 19, p. 328–330.

(12) MacIntire, W. H., Willis, L. G., and Holding, W. A. 1917 The divergent effects of lime and magnesia upon the conservation of soil sulfur. *In* Soil Sci. v. 4, p. 235.

(13) MILLER, N. H. J. 1905 The amounts of nitrogen as ammonia and as nitric acid, and of chlorine in the rainwater collected at Rothamsted. *In* Jour. Agr. Sci., v. 1, p. 280–303.

(14) PECK, E. L. 1917 Nitrogen, chlorine and sulfates in rain and snow. *In* Chem. News, v. 116, p. 283.

(15) SHAFFER, S. 1921 Substances dissolved in rain and snow. *In* Mon. Weath. Rev., v. 49, p. 404–405.

(16) STEWART, R. 1920 Sulfur in relation to soil fertility. *In* Ill. Agr. Exp. Sta. Bul. 227, p. 107.

(17) TRIESCHMANN, J. E. 1919 Nitrogen and other compounds in rain and snow. *In* Chem. News, v. 119, p. 49.

(18) WILSON, B. D. 1921 Sulfur supplied to the soil in rainwater. *In* Jour. Amer. Soc. Agron., v. 13, p. 227.

(19) WITUYNJ, J. 1911 Amounts of chlorine and sulphuric acid in rainwater. *In* Jour. Exper. Landw., v. 12, p. 20–30 (In Russian); *abs. in* Jour. Chem. Soc., v. 100, p. 432.

SOIL EXPERIMENT FIELDS AND THEIR VALUE

PERCY EDGAR BROWN

Iowa State College

Received for publication, January 30, 1922

The chief purpose of this paper is to give a little of the historical background for soil experiments, to call attention indirectly to the value of such tests as have been conducted in the past, and to illustrate certain important phases of such work by the results of experiments on Iowa soils. It has not been deemed necessary to go into the value of the work done in the past, to any large extent, for a mere reference to the experiments is sufficient in most cases to lead to very definite conclusions regarding their value. It may be said however, that if any consideration at all is given to the importance of making soil experiments of interest and value to farmers, the importance of field tests is certainly evident, and if the farm practices of today are compared with those of a few years ago, the value of the work which has so completely changed many farming operations is seen to be very great.

HISTORICAL

The details of the earliest field experiments on soils lie buried in the mists of antiquity but a study of agricultural history leads to the conclusion that such experiments have probably been carried out in a rude way from time immemorial.

The writings of Moses indicate that agriculture is as old as man and while specific information regarding the practices followed is lacking, it would seem that Isaac and Jacob, and probably Abraham also, tilled their fields and raised their crops by methods which they had found by experience and comparison to be the best. Apparently Isaac knew how to grow corn for it is said he reaped an hundredfold. What is more reasonable than to conclude that he learned by field experiments how to secure such large yields?

The first definite record of the application of manures to soils appears in the agricultural literature of the Greeks. Pliny ascribes the "invention" of manures to the Grecian king, Augeas. In Homer, an old king is found manuring his field with his own hands. These legends may very well be interpreted to mean that the value of manure was discovered through field experiments. Theophrastus, a disciple of Aristotle and often called the father of botany, wrote on the history of plants and his work contains many interesting observations on soils and manures. He enumerated six different kinds of manures and stated that a mixture of soils (sand with clay and vice versa) produced the same effects as manures. Would it not be quite logical to conclude that he had tested the accuracy of this statement in field experiments? Perhaps he and neither Abraham nor the Grecian king was the first soil experimentalist.

Much of the knowledge of this early period of the world's history is based upon conjecture and the speculations indulged in here are at least supported by as much of fact and definite evidence as the conclusions reached by historians along many other lines. Sufficient has been said to indicate that very early in the history of the world field experiments were probably an integral or at any rate a casual part in the operations of the leading agriculturists.

With the advent of the group of learned Romans who were interested in agriculture and whose writings form the foundation of all agricultural literature, conjectures are replaced by rather definite information, mere opinions by facts. The rise of the Roman Empire wrought fundamental and far-reaching changes throughout a large part of the world and in all phases of human activity. Agriculture along with the other arts and sciences was affected to only a slightly less extent than were governments, society and general living conditions. Indeed from the writings of Cato, Varro, Columella, Virgil, Pliny and Palladius it would appear that agriculture passed from the stage of an occupation to an art and perhaps even became a "fad" to some extent. De Re Rustica, the oldest Roman work on agriculture tells of the use of manure, of the plowing under of lupines as green manures, of the application of burned lime, and of the utilization of marl in Britain and Gaul with beneficial effects on the growth of crops. This information and much else of interest and value to farmers could have been secured only through the carrying out of field tests. There can be no question but that many tests were conducted on Roman farms to the lasting benefit of the art of agriculture.

The literature of this period and that of mediaeval times is not only filled with practical suggestions but it also contains many ingenious speculations, some of which have been found, by later work, to be sound and others of which have been shown to be fallacious. But it is not intended to consider here the various theories of soil fertility which have been suggested nor the development of our knowledge of the nutrition of plants. These have come as a result of the activities of the agricultural chemists whose investigations followed close upon the speculations of the Roman and mediaeval eras. Under their leadership agriculture entered upon a new and intensive period of development. Then there began the real experimental work, the results of which have formed the basis for the extensive investigations of recent years.

The years of activity on the part of the agricultural chemists have been divided into two main periods by Russel, characterized as The Search for the Principle of Vegetation, 1630–1750, and The Search for Plant Nutrients, 1750–1860. The latter period is subdivided into the Phlogistic Period, 1750–1800, and the Modern Period, 1800–1860. Passing over the earlier years, which were characterized by the brilliant investigations of such well-known scientists as Glauber, Jethro Tull, Francis Home, Priestley, Ingen-Housz, de Saussure, Davy and numerous others, all of whom carried on their studies in the laboratory or in small pots, it appears that, in 1834, Boussingault began a series of field experiments on his farm at Bechelbronn in Alsace—the first really scientific field tests. These experiments of Boussingault were so comprehensive and yielded results which were so striking that they have become almost classic. If the idea of field tests in ancient times cannot be accepted, at least it is certainly unnecessary to look further than Boussingault's work for evidence in support of the theory of their value. Perhaps the earlier studies in Palestine, in Egypt, in Greece and in Rome should not be dignified by the title of experiments but no one can question the experimental nature of Boussingault's investigations. An interesting digression might be made here to consider just what constitutes an experiment, a trial, and a demonstration, but the question is not essential to the subject in hand.

Only a few years elapsed until the idea of field experimentation led to the establishment of the world-famous Rothamsted Experiment Station. A few experiments were begun in 1837, on the farm of Sir John Bennett Lawes, more were added in 1840 and in 1843, the experimental work was officially begun under the direction of Lawes and his associate, Sir James Henry Gilbert. The plan of these field studies was based upon the earlier laboratory and pot culture work of many investigators but was influenced to the largest extent by the work of de Saussure and by the theories of Liebig, just enunciated. Hence the work centered around the mineral constituents of plants and particularly nitrogen nutrition.

The scope of the experiments at Rothamsted is so well known that nothing need be said about it here except to emphasize the fact that so many problems of practical as well as technical value have been studied there that practically all of the soil investigations which have been carried on in recent years have been based upon or at least suggested by the Rothamsted work. These investigations have been carried on continuously from 1843 to the present time and their value becomes greater with each succeeding year. The idea of the value of long-time field experiments on soils which quite generally prevails at the present time, needs no further support than that given by the Rothamsted fields.

Nine years passed before the first German experimental field was established at Möckern (Leipzig) and many more years elapsed before the fields in this country were laid out; but the ideal for all field tests in each case has been to imitate the Rothamsted experiments with such modifications as are necessary to fit the local conditions.

THE PRESENT SITUATION

The idea of the value of the Rothamsted work has become so firmly implanted and as a consequence soil investigators have become so accustomed to accepting long-time field studies as the most desirable and conclusive of all experimental work, that it is somewhat of a shock to read in a recent publication (1) that "it is highly probable that no fertilizer experiment as ordinarily conducted is possessed of sufficient practical value to justify the large expenditure of money, time and energy involved." The statement is made as a result of studies of the Pennsylvania and Ohio field experimental data and from a consideration of the "enormous variability of all soils and plants." The question immediately arises whether or not the statement is warranted. It demands careful attention from all who are interested in field experiments. Is the money which the national and state governments put into such work being wasted? Have the teachings of the past decade been all wrong? Are the farmers, the legislators and the scientists all being deceived?

It is not intended here to defend the Ohio and Pennsylvania experiments, not to call attention to the various questions which have been answered by them and by many other field investigations particularly those at Rothamsted. Neither is it intended to defend the interpretation of any of the data from these tests. After all, it is the interpretation of the data which is really attacked for the value of the field experiments lies mainly in the conclusions which are drawn from the results secured. When the first shock from the very startling statement given above has passed, the reaction which it brings about will undoubtedly be of distinct value from the standpoint of improving soil experiments. In other words, extreme views and statements frequently accomplish much good even if they are found to be too radical, for they stimulate study, thought and investigation, and in this case the sweeping condemnation of all field experiments will certainly cause more care to be taken in planning and carrying them out and in interpreting the data.

The field experiments of the past need no defense, they need study and the conclusions drawn from them should be utilized in present day work. Without question there have been weaknesses in the plan of experiments, faults which could not be foreseen, difficulties which could not be overcome, and conclusions have been drawn which were not entirely warranted. But assume all this to be true and the method of field experimentation still remains fundamentally sound. If it needs improvements, those improvements will come as a result of study. The Agronomy Society has a special committee collecting and tabulating all available information regarding field experiments and eventually the Society will adopt certain definite standards for such work. This will be a long step in advance and will make field experiments more valuable. The development of the soil survey work has yielded information regarding soil types which is bringing about desirable changes in the matter of locating experiment fields. Probably no other factor has been so completely overlooked, in the locating of fields as the selection of an area where the soil is of a definite type. In most cases expediency or nearness to the Experiment Station or a similar reason has controlled the selection of a field but the tendency now is to consider the soil type first and other considerations follow logically. Much more might be said along this line and other ways in which field experiments could be improved might be suggested but such a discussion is left for a later date when the recommendations of the Agronomy Society committee come up for final action.

It has been assumed that practically all will agree that the arraignment of field experiments is unwarranted and that there is no immediate likelihood of their discontinuance. Hence no defense of the principle of field experiments seems needed, other than the few comments which have been offered. The question of making such tests of the largest possible value is one however, which continually presses for attention.

IOWA EXPERIMENTS

A thorough study of the whole subject of soil experiment fields and their value was made when the Iowa Experiment Station began the rather extensive tests now under way. An attempt was made to profit by the experiences of the past, to modify plans to fit local conditions, and to get away as far as possible, from the objectionable features of the older fields. Just how the experimental data will be interpreted, what the outcome of the work in general will be, cannot now be predicted. Some results secured at the present time however, may be cited to indicate the effects of certain fertilizers on individual soil types under general farming conditions, and a comparison of the results from various fields will show the effects of the soil type on the results, the seasonal effects are shown in the results in different years and the general value of these field experiments to Iowa farmers may be apparent from the nature of the results.

The general plan which has been followed in the Iowa soil experimental fields has been to locate a series of plots in various parts of the state on individual soil types. These fields have been laid out following the survey of a county and the location has been very carefully selected, every precaution being taken that the soil be true to type, that it be true to typical topography of the type, that its past history be such that there be no interference from such factors as fence rows, manure piles, straw stacks, etc. Not only are the soil conditions very carefully selected but the cooperator is also carefully chosen. The aim is to secure an owner preferably and a man who is interested, willing to cooperate, and permanently located as far as can be determined.

TABLE 1

The effect of applications of manure on crop yields on individual soil types in Iowa

EXPERIMENT FIELD	COUNTY	SOIL TYPE	CROP	YEAR	YIELD ON CHECK PLOT	YIELD ON MANURE PLOT
					bu. or tons	*bu. or tons*
Waverly No. 2	Bremer	Carrington loam	Corn	1918	38.5	54.0
Waverly No. 2	Bremer	Carrington loam	Oats	1919	39.8	49.3
Clarinda	Page	Marshall silt loam	Corn	1920	54.3	64.4
Agency	Wapello	Grundy silt loam	Oats	1919	44.9	62.2
Agency	Wapello	Grundy silt loam	Wheat	1920	22.7	31.5
Everly	Clay	O'Neill loam	Clover	1921	1.80	2.35
Eldridge	Scott	Muscatine silt loam	Corn	1918	66.6	74.4
Eldridge	Scott	Muscatine silt loam	Oats	1919	43.2	50.7
Eldridge	Scott	Muscatine silt loam	Corn	1921	62.1	72.1
Eldora	Hardin	Carrington loam	Corn	1919	33.9	52.5
Eldora	Hardin	Carrington loam	Oats	1920	42.8	49.6
Jesup	Blackhawk	Carrington loam	Clover	1919	1.17	2.08
Jesup	Blackhawk	Carrington loam	Corn	1921	58.7	72.8
New London	Henry	Grundy silt loam	Clover	1916	2.10	2.90
New London	Henry	Grundy silt loam	Corn	1917	49.4	61.7
New London	Henry	Grundy silt loam	Oats	1920	32.0	49.8
Spencer No. 1	Clay	Carrington loam	Corn	1918	30.4	46.9
Spencer No. 1	Clay	Carrington loam	Oats	1919	39.9	51.0
Spencer No. 1	Clay	Carrington loam	Corn	1920	37.6	46.9
Storm Lake	Buena Vista	Webster silty clay loam	Corn	1920	48.2	57.3
Truesdale	Buena Vista	Carrington loam	Corn	1920	47.5	57.0
Truesdale	Buena Vista	Carrington loam	Corn	1921	32.8	39.7
Blue Grass	Scott	Muscatine silt loam	Clover	1920	1.57	1.92
Calamus	Clinton	Carrington silt loam	Clover	1918	1.45	3.04
Calamus	Clinton	Carrington silt loam	Corn	1920	57.5	65.2
Hudson	Blackhawk	Muscatine silt loam	Oats	1919	47.6	54.7
Hudson	Blackhawk	Muscatine silt loam	Corn	1920	53.2	62.8
Osage	Mitchell	Carrington silt loam	Oats	1920	34.6	60.3
Sherfey	Muscatine	Clinton silt loam	Corn	1919	53.5	74.8

The fields are located where a definite rotation is being practiced, whatever that rotation may be. The cooperator may be a livestock farmer or a grain farmer. In most instances the series of plots is so arranged that the tests include both types of farming and the results which are secured may yield valuable practical information regarding the relative merits of the two types of farming.

TABLE 2

The effect of lime on crop yields on individual soil types in Iowa

EXPERIMENT FIELD	COUNTY	SOIL TYPE	CROP	YEAR	YIELD ON MANURE PLOT	YIELD ON MANURE LIME PLOT
					bu. or tons	*bu. or tons*
Clarinda	Page	Marshall silt loam	Oats	1916	54.4	63.3
Clarinda	Page	Marshall silt loam	Clover	1917	1.36	1.56
Clarinda	Page	Marshall silt loam	Corn	1919	58.7	62.6
Clarinda	Page	Marshall silt loam	Oats	1920	52.3	61.8
Spencer No. 1	Clay	Carrington loam	Corn	1918	46.9	52.0
Spencer No. 1	Clay	Carrington loam	Oats	1919	51.0	59.7
Spencer No. 1	Clay	Carrington loam	Corn	1920	46.9	55.7
Alburnett	Linn	Clyde silt loam	Corn	1920	41.0	51.8
Alburnett	Linn	Clyde silt loam	Oats	1921	36.6	43.3
Blue Grass	Scott	Muscatine silt loam	Clover	1920	1.92	2.37
Blue Grass	Scott	Muscatine silt loam	Corn	1921	57.8	66.1
Waverly	Bremer	Carrington loam	Oats	1919	49.3	61.9
Waverly	Bremer	Carrington loam	Clover	1920	0.67	1.36
Mt. Pleasant 1	Henry	Grundy silt loam	Corn	1920	57.0	76.6
Mt. Pleasant 2	Henry	Grundy silt loam	Clover	1918	3.82	5.30
Mt. Pleasant 2	Henry	Grundy silt loam	Corn	1919	66.3	74.1
Mt. Pleasant 2	Henry	Grundy silt loam	Corn	1920	74.9	82.2
Sawyer	Lee	Grundy silt loam	Clover	1919	1.75	2.10
Sawyer	Lee	Grundy silt loam	Timothy	1920	3.48	4.17
West Point 1	Lee	Grundy silt loam	Oats	1919	41.5	43.5
West Point 1	Lee	Grundy silt loam	Clover	1920	1.87	2.89
Farson	Wapello	Grundy silt loam	Clover	1920	1.19	1.43
West Point 2	Lee	Grundy silt loam	Clover	1919	1.59	1.87
Eldridge	Scott	Muscatine silt loam	Corn	1918	74.4	81.8
Eldridge	Scott	Muscatine silt loam	Oats	1919	50.7	64.3
Low Moor	Clinton	Carrington silt loam	Timothy	1920	2.13	2.77

All the usual precautions are taken in locating the fields with regard to drainage, previous treatment of the soil, character of the crops grown and general method of farming practiced.

From preliminary studies it was determined that the soil problems of the greatest value in Iowa include the use of farm manure, the application of lime, the addition of phosphorus, either in the rock phosphate or acid phosphate form, the use of complete commercial fertilizers, and the turning under of crop residues. Hence the experimental fields have been planned to consist of 13 plots, part of which represent the livestock system of farming and test the use of manure with lime, phosphorus carriers and a complete fertilizer.

TABLE 3

The effect of phosphorus on crop yields on individual soil types in Iowa

EXPERIMENT FIELD	COUNTY	SOIL TYPE	CROP	YEAR	YIELD ON MANURE LIME PLOT	YIELD ON MANURE LIME ROCK PHOSPHATE PLOT	YIELD ON MANURE LIME ACID PHOSPHATE PLOT	YIELD ON MANURE LIME COMMERCIAL FERTILIZER PLOT
					bu. or tons	*bu. or tons*	*bu. or tons*	*bu. or tons*
Clarinda 1	Page	Marshall silt loam	Oats	1917	88.0	91.0	103.6	98.0
Clarinda 1	Page	Marshall silt loam	Clover	1918	1.20	1.75	1.50	1.70
Clarinda 1	Page	Marshall silt loam	Corn	1919	57.3	60.9	64.5	61.5
Newell	Buena Vista	Webster silty clay loam	Corn	1919	54.4	61.4	65.1	70.9
Newell	Buena Vista	Webster silty clay loam	Oats	1920	63.5	69.7	76.3	68.9
Orange City	Sioux	Marshall silt loam	Corn	1919	80.8	86.1	90.6	88.5
Orange City	Sioux	Marshall silt loam	Corn	1920	54.9	58.2	58.2	56.0
Storm Lake	Buena Vista	Webster silty clay loam	Corn	1920	58.1	64.2	76.5	80.0
Alburnett	Linn	Clyde silt loam	Corn	1920	51.8	61.5	63.0	83.3
Blue Grass 2	Scott	Muscatine silt loam	Clover	1919	2.94	3.06	3.06	3.16
Calamus	Clinton	Carrington silt loam	Clover	1918	3.38	4.39	5.18	4.35
Calamus	Clinton	Carrington silt loam	Clover	1919	0.74	1.22	2.11	1.66
Delmar	Clinton	Muscatine silt loam	Barley	1920	24.9	32.7	32.7	40.3
Eldora	Hardin	Carrington loam	Clover	1918	1.27	2.87	2.56	1.88
Eldora	Hardin	Carrington loam	Corn	1919	51.8	53.6	57.2	51.7
Hudson	Blackhawk	Muscatine silt loam	Corn	1920	67.4	73.3	73.3	72.4
Jesup	Blackhawk	Carrington loam	Corn	1918	40.4	52.7	50.3	59.2
Jesup	Blackhawk	Carrington loam	Oats	1919	40.5	43.5	52.4	58.9
Jesup	Blackhawk	Carrington loam	Clover	1920	1.10	1.57	2.20	2.00
Jesup 2	Blackhawk	Carrington loam	Clover	1919	1.86	1.92	2.22	2.80
Letts	Muscatine	Muscatine silt loam	Corn	1918	77.2	82.9	83.7	84.8
Letts	Muscatine	Muscatine silt loam	Oats	1919	58 6	62.6	62.6	67.4
Letts	Muscatine	Muscatine silt loam	Wheat	1920	23.9	28.5	27.5	31.2
Mount Joy	Scott	Muscatine silt loam	Alfalfa	1919	2.52	3.20	2.96	3.12
Waverly	Bremer	Carrington loam	Clover	1919	2 02	2.10	3.10	2.84
Waverly	Bremer	Carrington loam	Corn	1920	87.8	94.0	101.5	94.2
Mt. Pleasant	Henry	Grundy silt loam	Clover	1919	3.99	4.98	6.47	6.93
Mt. Pleasant	Henry	Grundy silt loam	Corn	1920	82.2	88.6	101.4	88.4
Rome	Henry	Clinton silt loam	Timothy	1920	0.95	1.63	2.85	1.63

The other portion of the series tests the use of crop residues instead of manure with the same fertilizers applied, this latter portion representing the grain system of farming.

There are some variations being made in new fields which have been established and in several of these additions of potassium are being made. With a few exceptions the field experiments of the state have been under way for a period of only five years and thus far yields have been secured for four seasons. Experience with field experiments teaches that data must be secured over a period of years before it should be interpreted, in order to take care of factors which unavoidably interfere with the results in individual years. Hence it is, of course, too early to draw definite conclusions from most of the Iowa fields. The data, however, is proving of considerable interest and value at the present time and it is possible, in fact, to draw from the results already secured certain conclusions of practical value to farmers. Some of the data secured during the past few years is included here in order to illustrate the results which are being secured from the use of the various fertilizing materials on individual soil types. These figures have been compiled to show separately the effects of manure, of lime, and of phosphorus fertilizers and are given in tables 1, 2 and 3. It is not intended to consider these tables in detail but merely to call attention to the practical conclusions to which they may lead, and to emphasize the significance of the results of such tests as planned and carried out in Iowa from the standpoint of increased crop production and permanent soil fertility.

It is always, desirable, of course to calculate all results on an economic basis and this method will be followed in future interpretation of the Iowa data. At present, however, it is felt that the data is not sufficiently complete to warrant such economic interpretation. In a few instances where calculations have been made, it has been shown that certain fertilizing materials increase the yield of certain crops on many individual soil types to a profitable extent. There is no reason to doubt but that the results secured in these experiments during the next few years will prove of large value to Iowa farmers and to the agriculture of the state.

In conclusion it should be emphasized that too much care cannot be take in planning and laying out field experiments. Some must unavoidably be relinquished—occasionally accidents will happen in making applications or securing results, but the data secured from such field tests over a period of years provides the only definite practical economic solution of the soil fertility problem of the individual farmer.

REFERENCE

(1) LIPMAN, C. B., AND LINHART, G. A. 1920 A critical study of fertilizer experiments. *In* Proc. Nat. Acad. Sci., v. 6, no. 11, p. 684.

SOME WAYS OF INCREASING THE DUTY OF IRRIGATION WATER

W. L. POWERS

Oregon Experiment Station

Received for publication January 30, 1922

Duty of water is perhaps the broadest problem with which the irrigationists have to deal. Duty-of-water data is of value to water boards and courts in determining water rights, to engineers in fixing capacities of canals and reservoirs, and to agriculturists in the control of soil moisture.

The amount of water needed for each of the chief soil classes and for each important group of crops varies according to their water requirement, average yields, and relative acreage on each project. Meadows constitute 52 per cent of the irrigated land on all government projects according to the crop census of the United States Reclamation Service. Meadow crops require about twice as much water as do annual crops, hence they deserve about 75 per cent of the consideration in fixing an average duty of water for a given area.

The amount of water provided affects the estimates and final cost, determines the area it is possible to irrigate, and has its effect upon the security of investment in irrigation and the ultimate agricultural and financial success of a project. To secure economical use of water on an irrigation project and avoid alkali and drainage problems there must be a good distribution system and each farmer must use water intelligently.

Too little water results in crop shortage, while excessive irrigation may lessen the crop and injure the soil in places to the point of unprofitable production. It is better economy to provide only a moderate allowance of water with reasonably priced structures than to provide a liberal supply at a greater expense and invite drainage assessments later on. The aim should be to get the highest practicable efficiency out of every acre-inch of rainfall and every supplementary inch of irrigation water provided. The cost of water that will give the greatest net profit an acre will generally represent the limit of preventable loss under present economic conditions.

A score of different factors affect the duty of water. Those affecting storage, conveyance and distribution losses and soil and crop requirements have been discussed in a previous paper (2). It is the purpose of this paper to present some additional data bearing on a few of the chief ways of increasing duty of water.

USABLE WATER CAPACITY OF SOILS

The field capacity of soils for storing usable soil-moisture affects the amount and frequency of irrigation and total quantity required per season very much. In duty-of-water studies it has often been found that farmers will apply six or eight inches of irrigation to a soil which has capacity to retain not over four inches. The surplus removes valuable plant foods, may cause drainage problems on adjacent low lands and involves needless expense.

Field water-capacity tests have been made in Oregon in connection with irrigation investigations for the leading soil types in the chief irrigated sections of the State. Duplicate tests are made using sheet metal cylinders 1 foot long and 6 inches in diameter. These are forced into the moist soil their entire length and are then dug out, struck off flush at the bottom and covered with a perforated base. These cores are then saturated, drained to constant weight in the covered jars, and their water retaining capacity determined by oven drying samples of the core. Other moisture points such as drought point and excess points are determined by methods described elsewhere (1). From these studies a generalized chart, figure 1, has been prepared as a guide to irrigators showing the usable water capacity of soil classes.

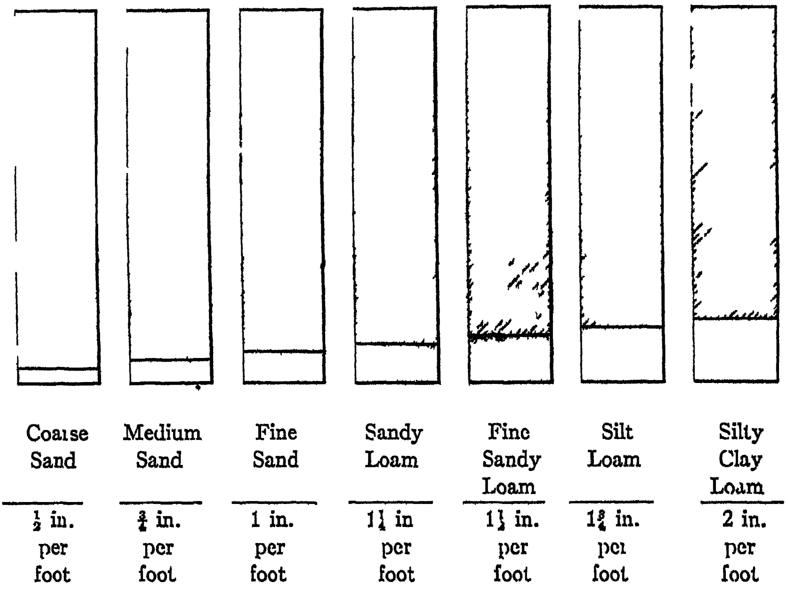


FIG. 1. USABLE WATER CAPACITY OF SOIL CLASSES

Modern irrigation contemplates that water be measured and applied in known quantities according to capacity of the soil and extent of the root system of the crop. Then to irrigate an acre of potatoes in which the feeding roots are largely within the first three feet of soil, on a fine sandy loam soil, having usable water-capacity of one acre-inch to the foot, three acre-inches will be required, provided the soil is dried out to the drought point. Since a cubic foot per second will make an acre inch in an hour it will require three hours with such a flow to irrigate the patch. The writer has shown elsewhere (3) that the wilting point has an important bearing on the best time to irrigate to obtain highest yield and duty of water.

VALUE OF ROTATION AND MANURE

Rotation of crops costs little and is very profitable on all soils. It permits increasing the humus and nitrogen supply by the turning under of legume sod and manure and crop residues thus increasing the water capacity, tilth and fertility. A 3-year rotation at the Oregon Experiment Station has, in 9 years, averaged a yearly net profit under irrigation of $14.09, while rotation and manure under irrigated conditions has increased the net profit an acre from beans by $23.02. The detailed data are presented in table 1. The rotation used was grain, clover, and beans. The yield and net profit per acre-inch are about doubled by the rotation and manure. The water requirement per pound of dry matter is reduced by these treatments nearly one-half. This water requirement is determined by sampling the soil at time of emergence of the plants and again at harvest, determining the soil moisture of each sample, and adding to the difference in pounds per acre the pounds of rain and irrigation water. The dry matter in the crop at harvest time was also determined so that, water-duty might be exactly measured as the ratio of water consumed to dry crop produced.

TABLE 1

Value of rotation—nine year average

TMENT	AVERAGE YEARLY YIELD		AVERAGE NET PROFIT			WATER REQUIREMENT PER POUND DRY MATTER
	Per acre	Per acre inch	Per acre	Grain from rotation or manure	Per acre inch	
	bu.	*bu.*				*lbs.*
Beans:						
Continuous unirrigated	9.24		$11.55			2909
Rotated unirrigated	10.13		20.37	$8.82		2249
Rotated and m red unirrigated	12.91		21.26	9.71		1900
Irrigated continuous	9.85	3.82	15.37		$5.12	2622
Irrigated and rotated	15.74	6.10	29.46	14.09	9.82	1794
and manured	18.29	7.14	38.39	23.02	12.80	1425

CROP PRODUCING POWER OF WATER

The water requirement has been determined for about sixty crop-plots each year for the past dozen years in connection with duty-of-water trials at the

Oregon Station (2). By hand picking each year the plat of each crop which gave the maximum net profit an acre and calculating the average water requirement of the best paying plat by years the twelve year average water requirement for the most profitable irrigation with good modern methods of farming has been obtained. This figure is given in average inches for different crops in figure 2.

It has taken 5.23 acre-inches to make a ton of dry alfalfa under good conditions as a twelve year average. This represents the net duty of water for this crop. That is, the duty of water is not likely to be less than fifteen to eighteen inches in a three-ton country or thirty to thirty-six inches in a section capable of producing 6 tons an acre a year.

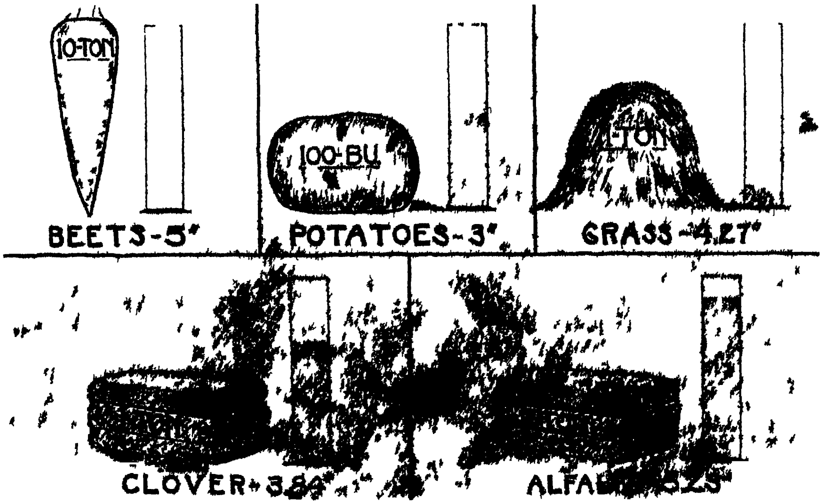


Fig. 2. Water Requirement of Crops on Plots Returning Maximum Net Profit Each Year, Based on a 12-Year Average

Potatoes, similarly, are not likely to be produced with less than three acre-inches per hundred bushels.

RELATION OF FERTILITY TO WATER REQUIREMENT

As indicated in the last column of table 1 the fertility of the soil affects the amount of water required per pound or per ton of dry crop produced. This was recognized and reported by the writer as early as 1912 and has been noted by others (1). Desiring more exact data on the matter a tank experiment was arranged to determine the water requirement of oats grown on two soils with different fertilizer treatments. One soil of strong fertility gave moderate variations in water cost with the different treatments, the other a soil of medium fertility, gave a striking difference in yield and water requirement (table 2). Other data published and unpublished from the Oregon Station substantiates the importance of fertility as affecting water requirement and duty of water. The richer and better balanced nutrient solution gives a lower water requirement.

The irrigation requirement and water requirement will vary somewhat with the season and anything which affects the evaporation, percolation or transpiration of soil moisture. Anything that contributes to good irrigation-farming such as planting, irrigating or cultivating at the right time, tends toward economical use of irrigation water. Proper economical irrigation is necessary to permanent irrigation agriculture. It is fundamentally important in irrigation farming to practice a careful rotation of crops that will permit plowing in legume sod and crop residues every few years to keep up water capacity and fertility and lower the water requirement. If we can save 50 per cent of the water used in many places we can practically double the crop producing power of such areas. Furthering the economical use of water will, therefore, help to establish highest productive values and add permanently to the food output and wealth producing area.

TABLE 2

Relation of fertility to water requirement

Oats on Deschutes medium sandy loam soil

Fertilizer	Amount per acre	Yield	Water requirement per pound of dry matter
	lbs	*gm.*	*lbs.*
Untreated	0	16.2	2125
Sulfur	100	57.9	594
Potassium chloride	160	37 5	1020
Tri-calcium phosphate	100	35.9	1034
Sodium nitrate	100	44.8	842
Sulfur	100	42.3	867
Sodium nitrate	100		
Sulfur	100	40.7	946
Potassium chloride	160		
Sulfur	100	29.2	1202
Tri-calcium phosphate	100		
Sodium nitrate	100	58.5	650
Potassium chloride	160		
Tri-calcium phosphate	100		
Untreated	0	15.0	2219
Manure	10 tons	76.3	390

The problem of economic duty of water is admittedly complex, but all the agricultural wealth developed and undeveloped in the arid West will be favorably affected by its proper determination. The cost of investigations would be returned many fold by security gained from water litigation.

REFERENCES

(1) Powers, W. L. 1916 Economical use of irrigation water. Ore. Exp. Sta. Bul. 140.

(2) Powers, W. L. 1920 Duty of water in irrigation. Ore. Exp. Sta. Dept. Soils. Bul. 161.

(3) Powers, W. L. 1922 Water capacity and drought point of soils. *In* Soil Sci., v. 14, p. 159–165.

A STUDY OF THE CAUSES OF FROST OCCURRENCE IN MUCK SOILS

GEORGE BOUYOUCOS AND M. M. McCOOL

Michigan Agricultural Experiment Station

Received for publication January 30, 1922

INTRODUCTION

It is a very general observation that when a rather light frost occurs during the growing season, frost-susceptible plants growing on muck and peat lands are almost invariably injured, while the same plants growing on mineral soils, adjacent to and on the same level as the muck and peat lands, are generally not touched. Our observations and investigations for a period of years with corn and certain truck crops have confirmed this general belief. Very often the boundary line between the organic and mineral soils is distinctly shown by the line of frozen plants. It has even been observed that where the muck or peat has been burned the plants grown on it escaped a frost while those on the unburned peat and muck were killed.

Up to the present time there appears to be no definite and satisfactory explanation for the phenomenon; various notions and hypotheses exist, but there seems to be no experimentally proved explanation. A true and definite explanation of the phenomenon is of the greatest practical importance, since the large areas of muck and peat lands in the United States constitute so important a portion of the crop-producing land of the country.

The Soils Department of the Michigan Agricultural Experiment Station inaugurated in 1911 a study of the soil temperature. Results of these investigations have already been reported (1, 2). In 1918 a study of the causes of frost occurrence in muck and peat lands was also commenced, and it is the purpose of this paper to present a preliminary and very brief report of a portion of the results obtained from this investigation thus far. At a later date it is hoped to present a detailed and complete report.

There are four principal factors which may be responsible for the difference in frost occurrence between organic and mineral soils. These are difference in color, difference in temperature of the air at various elevations, difference in specific heat by volume of soil, and difference in heat conductivity.

It is an accepted fact in physics that color has a very marked effect upon radiation as well as upon the absorption of heat. It is claimed that radiation and absorption are closely related, that the best absorbers are also the best radiators, and the poorest absorbers the poorest radiators. A black substance, for instance, is able to absorb practically all the heat rays on account of its low reflection, low diffusive power and high absorptive power, is a good heat absorber and also a good heat radiator. The opposite is true of a white substance. A white substance on account of its high reflective, high diffusive power and low absorbing power, is a poor absorber and a poor radiator.

Some soil physicists have concluded, therefore, that the black soils attain a very high temperature during the sun insolation, but that their temperature during the night is about the same if not lower than that of the light-colored soils on account of greater radiation.

It has been shown (1), however, that color has no effect upon radiation in darkness. From a theoretical consideration it seems that it should not, because all colors emit in dark rays of low refrangibility which are the same, while all the rays they absorb from the sun are of high refrangibility and are different.

Color, therefore, is probably not the factor responsible for the difference of frost occurrence between organic and mineral soils.

Difference in air temperature due to elevation and air drainage cannot be considered as an operating factor since the plants observed in these studies were growing on mineral soils adjacent to and on exactly the same elevation as the muck and peat soils.

The third factor has to do with the specific heat of soils by volume. Under natural and field conditions we find that muck and peat, on account of their large water content, possess a much greater specific heat by volume than do the mineral soils. This would, however, favor the organic rather than the mineral soils in the prevention of frost. Specific heat by volume is, therefore, probably not the responsible factor.

The fourth factor is the difference in heat conductivity between the organic and mineral soils. It is a well-established fact, of course, that under field conditions peat and muck conduct heat at considerably lower rates than the mineral soils. The results presented in this paper indicate that this factor is probably responsible, at least under normal conditions, for the difference in frost occurrence on the organic and mineral soils.

PLAN OF INVESTIGATION

In order to study the influence of the factors just discussed, an investigation was arranged and prepared as follows:

On a low and level bottom land consisting wholly of muck and peat several feet deep, a pit about 8 feet square and 6 feet deep was dug. On a neighboring hill, only about 200 feet distant but about 40 feet higher in elevation, a similar pit was dug. The soil of this hill consisted of clay loam. The pit on the muck land was filled with the silty clay from the hill and the pit on the hill was filled in turn with the muck soil from the muck land. In both cases the soils were thoroughly and carefully compacted and considerable time was allowed for them to settle before the experiment was commenced. Close to the pit containing the mineral soil a small tract of muck land was divided into three strips and treated differently.

The soil in one strip was maintained compact, in the second cultivated and in the third compact and covered with a thin layer of sand. Likewise, close to the muck pit on the hill, a strip of the native soil was kept compact and free of vegetation.

The temperature of the soils was measured directly at the surface, about 1 inch above the surface and at 6 inches below the surface. Air temperatures were taken at about 1 inch and at 6 feet above the soil surface.

Standard minimum thermometers were employed for the measurement of the temperature of the air and of the surface soil, while special mercury thermometers were used for the temperature of the soil at lower depths.

It is well recognized, of course, that it is not easy to determine the temperature of a gas, because the thermometer does not indicate the temperature of the surrounding medium, but the temperature of its own bulb. If a thermometer is surrounded by an opaque fluid or solid, it takes up heat by conduction from the surrounding medium and thus indicates the temperature of that medium. If the bulb of a thermometer is exposed in a gas, however, its temperature is determined both by the conduction of heat to or from the surrounding medium and the differences between the radiant energy absorbed and emitted by the thermometer.

In order to measure, as closely as possible, the real temperature both of the air and of the surface soil, each thermometer used for measuring the air temperature was housed in a shelter exactly like those used by the United States Weather Bureau. It consisted of a rectangular box about 20 inches long, 20 inches high and 10 inches wide, with a sloping roof, closed bottom and latticed sides. This box, with the thermometer inside, was placed right on the surface of the ground or at a height of 6 feet.

The thermometers for measuring the surface soil temperature were placed on the surface and their bulbs were covered with a very thin layer of soil.

For measuring the influence of the soil temperature on that of the air immediately above the soil, two procedures were followed. One consisted of placing the minimum thermometer about one inch above the surface soil and leaving the bulb entirely unprotected and exposed. The second consisted of protecting or screening the thermometer by placing it in one of the boxes described above from which the bottom had been removed. The temperature inside of the box, then, was influenced by the temperature of the soil. At the same time, however, the box served to screen or protect the thermometer against direct radiation into space.

EXPERIMENTAL RESULTS

Experimental results and field observations have been gathered for the last three years upon the subject, but since it is intended to publish these data in a rather detailed report later, only a few typical results will be presented here at present.

In table 1 are shown the temperature records of the whole experiment as described, for the night of October 5, 1921. This was the first night that frost occurred in the fall of 1921 for this region. Table 2 shows the temperature of the soils during the day preceding.

An examination of the results in table 1 reveals some most interesting and remarkable differences in temperature in the various soils and in the air. In the first place, they show that the temperature of the air varies greatly with the elevation.

In the second place, the data show that the clay loam had the highest surface minimum temperature, followed by sand-covered muck, compact muck and cultivated muck, in order. The surface temperature of the clay loam at the bottom land was 36.2°F., that of the sand-covered muck 31.8°, compact

TABLE 1

*Minimum temperature of air and soils for the night of October 5, 1921**

POINT OF MEASUREMENT		TEMPERATURE	
		On bottom land	On hill
		°*F.*	°*F.*
Air	6 feet above ground	32.0	35.0
	1 inch above surface	29.0	33.5
Clay loam compact	On surface	36.2	35.5
	1 inch above surface (thermometers in box)	34.2	35.0
	6 inches deep	46.5	47.0
Muck compact	On surface	31.0	32.0
	1 inch above surface (thermometers in box)	31.8	34.5
	6 inches deep	50.5	52.1
Muck cultivated	On surface	28.0	
	1 inch above surface (thermometers in box)	30.5	
	6 inches deep	51.4	
Muck, compact and covered with layer of sand	On surface	31.8	
	1 inch above surface (thermometers inb ox)	32.8	
	6 inches deep	49.8	

* These results represent single determinations but they are typical of a great number of measurements made during four years.

TABLE 2

Maximum temperature of soils at 1 and 4 P.M. during the day of October 5.

POINT OF MEASUREMENT		BOTTOM-LAND TEMPERATURE		HILL TEMPERATURE	
		1 p.m.	4 p.m.	1 p.m.	4 p.m.
		°*F.*	°*F.*	°*F.*	°*F.*
Clay loam compact	On surface	55.0	57.0	56.0	58.0
	6 inches depth	54.1	54.0	54.0	54.8
Muck compact	On surface	58.0	57.0	57.0	58.0
	6 inches depth	51.2	53.1	52.7	54.0
Muck cultivated	On surface	57.0	57.0		
	6 inches depth	51.7	53.2		
Muck, compact and covered with layer of sand.	On surface	56.0	58.0		
	6 inches depth	51.8	54.0		

muck 31, and cultivated muck 28. The difference in temperature between the clay loam and the cultivated muck is 8.2°.

The same variation in temperature exists between the clay loam and the muck on the top of the hill. The temperature of the muck at this place is 32°, that of the clay loam 35.5°, or a difference of 3.5° in favor of the latter.

Now it is to be noted that while the temperature of the clay loam is higher than that of the muck at the surface, at the 6-inches depth it is the muck that has the higher temperature. It will be seen that when the minimum temperature was attained at the surface the temperature of the cultivated muck at the 6-inches depth is 4.9° and that of the compact muck 4° higher than that of the clay loam. Yet at the surface the temperature of the clay is 8.2° higher than that of the cultivated muck.

This difference cannot be due to any accumulated difference in the temperature between the various soils during the day, because the records in table 2 show that the temperature of all the soils at the various depths during the preceding day is about the same. Thus, at about 4 o'clock in the afternoon the temperature of the various soils at the corresponding depths is about equal.

The most apparent and logical explanation for the difference in temperature at the surface between the mineral and organic soils and consequently for the difference in frost occurrence between them, seems to lie in the difference of their heat-conducting power. The mineral soils are good conductors of heatl while the organic soils are comparatively poor conductors of heat. The mineral soils, therefore, allow the heat, which has been accumulated at the various depth, during the day, to travel to the surface at a greater speed than in the case of the organic soils. The result is that the surface of the mineral soil is kept at a higher temperature, while that of the organic soils is allowed to become excessively cold, even though their temperature at the lower depths is much higher than that of the mineral soils. The air above the mineral soils, therefore, is warmed to a correspondingly greater degree than that above the organic soils, and in a night when not too heavy a frost occurs, the mineral soils are able to prevent a frost, while on the organic soils frost occurs.

The effective influence of the soil-surface temperature on the air temperature above is strikingly illustrated by the results in table 1. It will be seen that the temperature of the air one inch above the clay is 34.2°F., while that above the cultivated muck 30.5°, or a difference of 3.7°.

This influence is further remarkably illustrated under actual field conditions. It has been abundantly observed that when a heavy frost occurs in the soil so that even the plants grown on the mineral soils are bitten by it, in the case of corn only the top portion is frozen, the lower portion, which is nearer to the influence of the surface-soil temperature is not at all touched, while the corn grown in the adjacent muck land is frozen from top to bottom.

It is to be further noted from table 1 that not only is the surface temperature of the muck lower than that of the mineral soil, but it is even lower than that of the air one inch above. Thus, the surface temperature of the cultivated muck is 28°F., while that of the air one inch above is 30.5°. On top of the hill it is 32° for the muck and 33.5° for the air. In the one case the surface of the mineral soil, however, is 2° warmer than the air one inch above.

The factor of heat conductivity, therefore, appears to be mainly responsible for the difference in frost occurrence on these soils. The agents or cultural practices that tended to accelerate the heat-conducting power of the soil, tended also to minimize the danger of frost occurrence. Packing of the soil and especially of the organic soils, and a high moisture content both help to increase the rate of heat transference of soils.

The influence of packing is very strikingly shown in table 1, where it is seen that the temperature of the compact muck at the surface is 31°F., while that of the cultivated is 28°, or a difference of 3° in favor of the compaction.

The influence of the water-content is well exemplified by the following practical observation. In the fall of 1920, corn grown in a basin of muck land where the drainage was very poor and the water-table and moisture-content high, was hardly touched by a frost, while the corn grown on the surrounding muck land with a slightly higher elevation and much drier, was completely killed by the frost. Professor Alway has related to us somewhat similar observations in Minnesota.

Referring once more to table 1, it will be seen that the surface temperature of the muck covered with a thin layer of sand is slightly higher than that of the compact muck. The cause for this difference is not altogether clear, but, probably it is due to some way in which the layer of sand favorably affects the heat conductivity of the muck. It may accomplish this by acting as a mulch and thereby maintaining a higher moisture content and by forming a more compact layer of soil at the surface.

The striking effect of the sand mulch on the temperature of the muck is further confirmed in a four years temperature measurement of gravel, sand, loam, clay and muck (2). These data show that when the muck was covered with a thin layer of sand its average temperature during the summer months was appreciably higher both at the 2- and the 12-inch depth than that of all the mineral soils. When not covered with the sand layer its average temperature was somewhat lower than that of the mineral soils. The higher average temperature in the former case was explained on the basis that the sand layer, acting as a mulch, diminished the water evaporation and the heat which would otherwise be expended in the evaporation of the water went to warm up the soil mass itself.

Another soil factor which appears to have an appreciable bearing on freezing of plants is the soil fertility. Various observations show that where soils have been rather heavily fertilized, corn or truck crops grown on them may sometimes escape a frost, while the same kind of plants grown on adjacent soil, but unfertilized, may be completely killed by the frost.

SUMMARY

The present paper is a preliminary report of an investigation on the causes of frost occurrence in muck soils. It is a general observation that when a frost occurs during the growing season, plants grown on muck and peat soils may be completely killed by the frost, while the same plants grown on mineral soils adjacent to and even on the same level, may not be at all touched by the frost.

Results obtained seem to show that heat conductivity is the predominant factor responsible for this difference in frost occurrence. The mineral soils are good conductors of heat, while the organic soils are comparatively poor conductors of heat. The mineral soils, therefore, allow the heat, which has been accumulated at various depths during the day, to travel to the surface at a greater speed than in the case of the organic soils. The result is that the surface of the mineral soils is kept at a higher temperature, while that of the organic soils is allowed to become excessively cold, even though their temperature at the lower depths is much higher than that of the mineral soils. The air above the mineral soils, therefore, is warmed at a correspondingly greater degree than that above the organic soils, and on a night when not too heavy a frost occurs the mineral soils are able to prevent a frost, while on the organic soils frost occurs.

REFERENCES

(1) Bouyoucos, George 1913 An investigation of soil temperature and some of the most important factors influencing it. Mich. Agr. Exp. Sta. Tech. Bul. 17.

(2) Bouyoucos, George 1915 Soil temperature. Mich. Agr. Exp. Sta. Tech. Bul. 26.

THE RELATION OF SOIL MOISTURE TO PHYSIOLOGICAL SALT BALANCE FOR PLANTS[1]

JOHN W. SHIVE

New Jersey Agricultural Experiment Stations

Received for publication February 17, 1922

INTRODUCTION

This paper deals primarily with soil moisture in relation to the physiological balance of the mineral elements required for plant growth. It presents the results of an experimental study of the growth of young buckwheat plants in soil cultures and corresponds to a somewhat similar investigation described in a previous publication (10) dealing with the growth of young wheat plants in sand cultures to which nutrient solutions were added in such a way as to produce different degrees of moisture in the cultures of the three different series employed.

As explained in the earlier paper, the purpose of these investigations was to determine in what manner the physiological balance of salt proportions (as these affect the growth of plants) is influenced by different moisture conditions of the substrata in which the plants are grown, and further to study the effectiveness, or the relative plant-producing value, of various combinations of the active fertilizer elements under different soil moisture conditions.

PLAN OF THE EXPERIMENTS

Buckwheat was grown in soil cultures for periods of four weeks. The soil used consisted of a homogeneous mixture of equal parts, by volume, of rich sandy loam and carefully washed, white, seashore sand. The soil was thus diluted with the sand in order that the mineral elements present in the medium might not too greatly obscure the effects of added salts upon the growth rates of the plants. The maximum water-retaining capacity of this mixture, determined according to the method of Hilgard (5) was 31.24 per cent on the dry-weight basis, this value being the average of five determinations. To the soil cultures prepared with the above described mixture the fertilizer salts were added in the form of solutions each of which contained the three salts, KH_2PO_4, $Ca(NO_3)_2$, and $MgSO_4$. The solutions used comprised a series of 21 different sets of salt proportions and are described as the solutions of type I in a "plan for cooperative research prepared for a special committee of the National Research Council," (6) but as here used the solutions had higher total concentration values than that specified in this plan.

The solutions were added to the air-dry soil in such quantities as to produce the same initial moisture conditions in all the cultures of a single series. Corresponding cultures of the various series were all alike with respect to the proportions of the salts added to the soil but differed from each other in the soil-moisture content. All the soil cultures of one series were prepared to have 30 per cent moisture based on the maximum water-retaining capacity of the soil, the cultures of a second series had a moisture-content of 60 per cent, and those of a third series had a moisture-content of 80 per cent on the same basis. The initial moisture-content of the cultures of the second series was chosen to approximate that required for optimum growth of the plants, while that of the cultures of the first and third series, respectively, was considerably above and below this optimum. All the fertilizer elements added to the soil were supplied in this initial application of the solutions to produce the required moisture-content of the soil cultures which was subsequently maintained by the frequent addition of distilled water to each culture in sufficient quantities to restore the moisture lost through transpiration. Evaporation from the surface of the soil was prevented by a wax seal (2).

---

[1] Paper No. 80 of the Journal Series, New Jersey Agricultural Experiment Stations, Department of Plant Physiology.

This paper will appear in Rutgers College Studies, vol. 1.

In creating the required initial soil moisture-content of corresponding cultures of the three series, as was here done by the use of nutrient solutions, it was not possible of course, to prevent the introduction of variable factors other than that of soil moisture and these must be considered in relation to the growth of the plants. The variable factors in question are those of total salts added per culture, total salts per unit volume of soil (involving total concentration of the soil solution), and the amounts of soil used per culture which depend upon the three possible methods of creating the required initial moisture conditions of the soil cultures by the use of nutrient solutions. These three methods were used in the present work which gave rise, unavoidably, to as many sets of experimental possibilities, each involving the three series of soil cultures above described.

The experimental possibilities, representing the unavoidable sets of cultural differences to which the plants may be subjected, with the given differences in the initial soil-moisture content in corresponding cultures of the three series, are set forth in table 1. The table is divided into three horizontal sections each of which give the data for a single group of cultures comprising the three series and representing one of the three sets of experimental possibilities. For the sake of convenience the groups are numbered consecutively I, II, and III and the series of each group will be designated the sub-optimal, optimal, and supra-optimal series according as the moisture-content of the cultures in the series was 30 per cent, 60 per cent, or 80 per cent of the maximum water-retaining capacity of the soil, respectively. The weights, in grams, of the air-dry soil used per culture, the amounts of solution in cubic centimeters added to the cultures of the different series to give the required moisture content, and the approximate total concentration values of the solutions, in atmospheres, are given. In the last two columns of the table are given the total salts per culture and the total salts per unit-volume of soil in terms of these values for the cultures of the optimal series taken as 1.00 in each case.

The methods used in creating the required initial soil-moisture and the soil conditions, with respect to other variable factors, resulting therefrom are briefly stated as follows: (1) Solutions alike in every respect were added in equal volumes to such amounts of soil as were inversely proportional to the required initial soil-moisture in corresponding cultures of the three series. The total salts per culture were then the same throughout and the total salts per unit volume of soil were directly proportional to the required soil-moisture in corresponding cultures. (2) Solutions alike in ever respect were added to equal amounts of soil in such volumes as were directly proportional to the required soil-moisture. The total salts per culture and the total salts per unit volume of soil were then also directly proportional to the required soil-moisture in corresponding cultures of the three series. (3) Solutions with total salt concentrations which were inversely proportional to the required soil-moisture, but alike in every other respect, were added to equal amounts of soil in such volumes as were directly proportional to the required soil-moisture. The total salts per culture as well as the total salts per unit volume of soil were then equal, respectively, in corresponding cultures of the three series. It is here assumed that the addition of the salts in the solution form to the soil of the various cultures was essentially the same in effect as adding the salts in the dry form to the soil and subsequently creating the required soil-moisture content by the addition of distilled water in the proper amounts.

The volume-molecular partial concentrations, which indicate also the molecular salt proportions of the 21 different solutions in each series of the three groups are given in table 2. The solution numbers refer to the positions which the solutions occupy in the triangular diagram representing the series as described in a "plan for cooperative research prepared for a special committee of the National Research Council," above cited.

In order to make the study as complete as possible the three groups of cultures as outlined in table 1, each group comprising the three series. were conducted simultaneously. Each series included in addition to the 21 treated cultures two check cultures which were prepared by adding distilled water instead of solutions to the air-dry soil in the proper amounts to produce the required initial soil moisture. It will be observed from the data of table 1 that all the optimal series were alike in every respect. These were, therefore, omitted from two of the groups in the actual experimentation leaving a total of seven series which were conducted simultaneously.

TABLE 1

Numerical data describing the initial treatment and condition of the soil cultures employed in groups I, II and III

SERIES	AMOUNT OF SOIL USED PER CULTURE	SOIL-MOISTURE BASED ON MAXIMUM WATER-HOLDING CAPACITY	TOTAL AMOUNT OF SOLUTION ADDED TO EACH CULTURE	APPROXIMATE CONCENTRATION OF SOLUTIONS ADDED TO CULTURES	RELATIVE VALUES BASED ON THOSE OF OPTIMAL SERIES TAKEN AS 1 00	
					Total salts per culture	Total salts per unit volume of soil
	gm.	*per cent*	*cc.*	*atmospheres*		
Group I:						
Suboptimal	3200	30	300	1.75	1.00	0.50
Optimal	1600	60	300	1.75	1.00	1.00
Supra-optimal	1200	80	300	1.75	1.00	1.33
Group II:						
Sub-optimal	1600	30	150	1.75	0.50	0.50
Optimal	1600	60	300	1.75	1.00	1.00
Supra-optimal	1600	80	400	1.75	1.33	1.33
Group III:						
Sub-optimal	1600	30	150	3.50	1.00	1.00
Optimal	1600	60	300	1.75	1 00	1.00
Supra-optimal	1600	80	400	1.31	1.00	1.00

Half-gallon glazed earthenware pots were used as culture-vessels except for the cultures of the sub-optimal series of group I which required larger pots and for those of the supra-optimal series of the same group for which somewhat smaller pots were used. The seedlings were carefully selected from a large number grown on germinating nets (9). Four seedlings were transplanted to each soil culture previously prepared. The cultures were then continued until all the plants were in full bloom which required a period of 28 days from the time of transplanting.

Throughout the growth period, excessive changes in the moisture-content of the soil through the loss of transpirational water were prevented by weighing the cultures daily and supplying sufficient amounts of distilled water at each weighing to restore the original moisture-conditions. During the last two weeks of the growth period the cultures were weighed twice daily and the original moisture conditions restored, unless the atmospheric conditions of the greenhouse in which the cultures stood were such as to permit only very low rates of transpiration. Under such conditions distilled water was addded to the cultures only once daily. The maximum loss of water from any culture of the three groups during any one interval between two successive weighings did not exceed 1.25 per cent, on the dry-weight basis. The average loss of transpirational water per culture during the intervals between successive weighings was, of course, very much lower than this and the loss was sustained during a very short period of time only. Thus the soil-moisture content of the cultures throughout the entire period of growth was maintained within very narrow variation limits.

TABLE 2

Volume-molecular proportions of KH_2PO_4, $Ca(NO_3)_2$, and $MgSO_4$ in the solutions added to the soil cultures in the proper amounts to create a soil-moisture content of 30 per cent, 60 per cent, and 80 per cent of the maximum water-retaining capacity of the soil in the sub-optimal, optimal, and supra-optimal series, respectively, in the different groups

SOLUTION NUMBER	VOLUME-MOLECULAR PROPORTIONS								
	Solutions used with the sub-optimal, optimal and supra-optimal series of groups I and II, and with the optimal series of group III (Concentration 1.75 atm.)			Solutions used with the sub-optimal series of group III (Concentration 3.50 atm.)			Solutions used with supra-optimal series of group III (Concentration 1.31 atm.)		
	KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$	KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$	KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$
R_1S_1	0.0047	0.0047	0.0282	0.0094	0.0094	0.0564	0.0035	0.0035	0.0211
S_2	0.0044	0.0086	0.0215	0.0088	0.0127	0.0430	0.0033	0.0064	0.0161
S_3	0.0042	0.0124	0.0164	0.0084	0.0248	0.0328	0.0031	0.0093	0.0123
S_4	0.0039	0.0156	0.0117	0.0078	0.0312	0.0234	0.0029	0.0117	0.0088
S_5	0.0039	0.0189	0.0075	0.0078	0.0378	0.0150	0.0029	0.0142	0.0056
S_6	0.0035	0.0213	0.0035	0.0070	0.0426	0.0070	0.0026	0.0160	0.0026
R_2S_1	0.0093	0.0047	0.0281	0.0186	0.0094	0.0562	0.0069	0.0035	0.0173
S_2	0.0086	0.0086	0.0173	0.0172	0.0172	0.0346	0.0064	0.0064	0.0130
S_3	0.0082	0.0124	0.0124	0.0164	0.0248	0.0248	0.0062	0.0093	0.0093
S_4	0.0079	0.0158	0.0079	0.0158	0.0316	0.0158	0.0059	0.0118	0.0059
S_5	0.0072	0.0182	0.0037	0.0144	0.0364	0.0074	0.0054	0.0136	0.0028
R_3S_1	0.0133	0.0044	0.0177	0.0266	0.0088	0.0354	0.0100	0.0033	0.0132
S_2	0.0126	0.0084	0.0126	0.0252	0.0168	0.0252	0.0094	0.0063	0.0094
S_3	0.0119	0.0119	0.0079	0.0238	0.0238	0.0158	0.0089	0.0089	0.0059
S_4	0.0114	0.0151	0.0037	0.0228	0.0302	0.0074	0.0085	0.0113	0.0028
R_4S_1	0.0173	0.0044	0.0130	0.0346	0.0088	0.0260	0.0130	0.0033	0.0097
S_2	0.0165	0.0082	0.0082	0.0330	0.0164	0.0164	0.0123	0.0062	0.0062
S_3	0.0158	0.0119	0.0039	0.0316	0.0238	0.0078	0.0118	0.0089	0.0029
R_5S_1	0.0215	0.0042	0.0086	0.0430	0.0084	0.0172	0.0161	0.0031	0.0064
S_2	0.0206	0.0082	0.0040	0.0412	0.0164	0.0080	0.0155	0.0062	0.0030
R_6S_1	0.0254	0.0042	0.0042	0.0508	0.0084	0.0084	0.0190	0.0031	0.0031

The three sub-optimal series, one of the like optimal series, and the three supra-optimal series were conducted simultaneously during the 28-day period from November 13 to December 11, 1920 and these were then repeated during the period from March 10 to April 7, 1921. At the end of each period of growth the plants were harvested. The tops were dried at a temperature of about 102°C. and the dry weights obtained in the usual way. Owing to the great difficulty of accurately weighing roots that have grown in soil, the dry-weight yields of tops alone will here be considered.

EXPERIMENTAL DATA

The dry-weight values of tops are given in tables 3, 4, and 5. Each of the tables presents the data for the three series of cultures (one for each degree of soil moisture employed) comprised in a single one of the three groups. The culture numbers shown in the first column of each table define the positions which the cultures occupy in the series with respect to the triangular diagrams (6) employed to represent graphically the partial molecular concentrations and the molecular salt proportions of the solutions added to the soil-cultures to produce the required initial soil-moisture. Since the triple series of cultures in each group was repeated, the data in the tables represent averages of the yield-values obtained from corresponding cultures of like series conducted during different time-periods. The first column in each section of the tables representing the data for a single series, gives the average absolute yield-values in grams while the second column gives the average dry weight of tops relative to the corresponding weight of culture R_1 C_1 taken as 1.00. The relative data represent the values obtained by averaging the relative yield-values from corresponding cultures of like series conducted during different time-periods. These are, therefore, averages of two or more ratios and not the ratios of averages, which accounts for the fact that the relative values given in the tables do not always bear exactly the same relation to one another as do the absolute yield values.

As indicated in table 1, the optimal series in the three groups of cultures were identical. As previously stated, therefore, a single series sufficed to furnish the data for the optimal series in each of the three groups of cultures conducted simultaneously. This accounts for the fact that the average data for the optimal series in groups I and II (tables 3 and 4) are alike. It will be observed, however, that the data for the optimal series in group III (table 5) are not the same as are those of the corresponding series in groups I and II since the series of these two groups were only once repeated while those of group III were twice repeated. The data in tables 3 and 4 thus represent average dry-weights from corresponding cultures of two like series while those in table 5 represent average dry-weights from corresponding cultures of three like series.

The average relative data for the cultures which produced the highest seven yields in each series are shown in bold-face type in the tables. These values taken from the proper columns of tables 3, 4, and 5 were plotted on the triangular diagrams shown in figures 1, 2, and 3, respectively, as was done in the earlier publication (10) dealing with the relation of the moisture-content in sand-cultures to salt-balance for wheat plants and corresponding to the present work with buckwheat plants in soil-cultures. The shaded areas on these triangular diagrams show the distribution in the series of the seven high-yielding cultures (upper one-third) only, since medium and low yields are of little interest in this connection. The positions of the cultures which produced the maximum yields in their respective series are marked with circles.

TABLE 3

Average absolute and relative dry weights of buckwheat tops grown 28 days in soil cultures of group I to which were added nutrient solutions (all having an osmotic concentration value of approximately 1.75 atmospheres) in the proper amounts to produce a soil-moisture content of 30 per cent, 60 per cent, and 80 per cent in the cultures of the sub-optimal, optimal, and supra-optimal series, respectively

CULTURE NUMBER	DRY WEIGHTS (4 PLANTS)					
	Sub-optimal series, 30 per cent moisture		Optimal series, 60 per cent moisture		Supra-optimal series, 80 per cent moisture	
	Absolute	Relative to R_1C_1 as unity	Absolute	Relative to R_1C_1 as unity	Absolute	Relative to R_1C_1 as unity
	gm.		*gm.*		*gm.*	
R_1C_1	1.5690	1.00	2.0560	1.00	1.6297	1.00
C_2	1.9976	1.22	2.5179	1.19	2.3746	1.40
C_3	2.2424	1.38	2.6231	1.26	2.4892	1.46
C_4	2.2520	1.39	3.1569	1.53	2.5760	1.54
C_5	1.9819	1.19	3.2918	1.61	2.2452	1.31
C_6	2.0112	1.20	3.0663	1.42	2.1671	1.35
R_2C_1	1.2541	0.82	2.0655	1.07	1.6513	1.00
C_2	2.0513	1.20	2.3480	1.27	2.1439	1.26
C_3	1.9857	1.16	3.3113	1.58	2.4689	1.48
C_4	2.3186	1.32	3.1674	1.52	2.5505	1.55
C_5	2.2625	1.34	3.3878	1.60	2.5342	1.46
R_3C_1	1.5336	0.98	2.1167	1.17	1.5660	0.97
C_2	1.8676	1.14	2.4931	1.25	2.5581	1.50
C_3	1.9027	1.18	3.0160	1.55	2.2293	1.35
C_4	2.2863	1.33	3.3692	1.61	2.7488	1.63
R_4C_1	1.4102	0.90	2.0094	1.04	1.7790	1.11
C_2	1.8730	1.14	2.4301	1.19	1.6545	1.06
C_3	2.2431	1.35	2.7042	1.35	2.4897	1.40
R_5C_1	1.5447	0.98	1.9071	0.98	1.5825	1.00
C_2	1.8900	1.16	2.4248	1.27	1.8030	1.10
R_6C_1	1.6497	1.03	2.0310	1.10	1.5574	0.97
Check	0.8498	0.57	1.1010	0.56	0.4829	0.31

The three triangular diagrams showing graphically the distribution of the high-yielding cultures in the sub-optimal, optimal and supra-optimal series of any one of the three groups of cultures are brought together in a single figure for ready comparison. The three series in each group will be compared separately with respect to high yield-values and will then be considered in connection with the corresponding series in the other groups.

TABLE 4

Average absolute and relative dry weights of buckwheat tops grown 28 days in soil cultures of group II to which were added nutrient solutions (all having an osmotic concentration value of approximately 1.75 atmospheres) in proper amounts to produce a soil-moisture content of 30 per cent, 60 per cent, and 80 per cent in the cultures of the sub-optimal optimal, and supra-optimal series, respectively

CULTURE NUMBER	DRY WEIGHTS (4 PLANTS)					
	Sub-optimal series, 30 per cent moisture		Optimal series, 60 per cent moisture		Supra-optimal series, 80 per cent moisture	
	Absolute	Relative to R_1C_1 as unity	Absolute	Relative to R_1C_1 as unity	Absolute	Relative to R_1C_1 as unity
	gm.		*gm.*		*gm.*	
R_2C_1	1.3735	1.00	2.0560	1.00	2.1427	1.00
C_2	1.6917	1.21	2.5179	1.19	2.0607	1.00
C_3	1.6824	1.21	2.6231	1.26	2.4264	1.10
C_4	1.7928	1.22	3.1569	1.53	2.4070	1.17
C_5	2.0667	1.47	3.2918	1.61	1.8975	0.82
C_6	1.9132	1.27	3.0663	1.42	2.4261	1.15
R_2C_1	1.2926	0.96	2.0655	1.07	2.0307	0.99
C_2	1.6201	1.15	2.3480	1.27	2.2098	1.06
C_3	1.8601	1.30	3.3113	1.58	2.4720	1.21
C_4	1.9859	1.33	3.1673	1.52	3.0407	1.36
C_5	1.8811	1.31	3.3878	1.60	2.4330	1.23
R_3C_1	1.3002	0.94	2.1167	1.17	1.8600	0.93
C_2	1.6513	1.18	2.4931	1.25	2.0888	0.98
C_3	1.4628	1.05	3.0160	1.55	2.6455	1.30
C_4	1.9858	1.39	3.3692	1.61	2.6919	1.20
R_4C_1	1.3992	1.04	2.0094	1.04	1.8895	1.00
C_2	1.6309	1.15	2.4301	1.19	2.1084	1.12
C_3	1.7092	1.19	2.7042	1.35	1.9671	0.99
R_5C_1	1.4511	1.06	1.9071	0.98	1.7352	0.95
C_2	1.7836	1.26	2.4248	1.27	2.0095	1.08
R_6C_1	1.2687	0.92	2.0310	1.10	1.6023	0.78
Check	0.8455	0.57	1.1010	0.56	0.5953	0.35

Group I

The method by which the required soil-moisture in the cultures of this group was created has already been described together with the experimental condition of the cultures at the beginning of the growth period. As shown in table 1, the amounts of soil used per culture in the different series were made inversely proportional to the required soil-moisture so that, when equal amounts of the same solution were added to the proper amounts of soil to

TABLE 5

Absolute and relative dry weights of buckwheat tops grown 28 days in soil cultures of group III to which were added nutrient solutions, having total osmotic concentrations values of approximately 3.50 atmospheres in the sub-optimal 1.75 atmospheres in the optimal and 1.31 atmospheres in the supra-optimal series, in proper amounts to produce a soil-moisture content of 30 per cent, 6o per cent, and 80 per cent in the cultures of the three series, respectively

CULTURE NUMBER	DRY WEIGHTS (4 PLANTS)					
	Series A, 30 per cent moisture		Series B, 60 per cent moisture		Series C, 80 per cent moisture	
	Absolute	Relative to R_1C_1 as unity	Absolute	Relative to R_1C_1 as unity	Absolute	Relative to R_1C_1 as unity
	gm.		*gm.*		*gm.*	
R_1C_1	1.1615	1.00	1.1415	1.00	0.9790	1.00
C_2	1.2737	1.10	1.2600	1.11	1.1887	1.21
C_3	1.0305	0.88	1.3668	1.20	0.9008	0.92
C_4	1.1675	1.00	1.6954	1.49	1.0413	1.07
C_5	1.1160	0.95	1.7920	1.60	1.1167	1.14
C_6	1.1430	0.98	1.4160	1.24	1.1612	1.19
R_2C_1	1.1510	0 99	1.3729	1.20	0.9468	0.95
C_2	1.2707	1.09	1.4972	1.30	1.1113	1.14
C_3	1.1846	1.02	1.6608	1.48	1.0913	1.12
C_4	0.9748	0.82	1.6636	1.48	0.9778	1.00
C_5	1.2633	1.09	1.6962	1.49	0.9384	0.95
R_3C_1	1.1388	0 97	1.6328	1.45	0.9722	1.00
C_2	1.2380	1.07	1.4750	1.31	1.0060	1.03
C_3	1.3782	1.19	2.0045	1.75	1.2420	1.17
C_4	1.3060	1.13	1.7174	1.51	1.0315	1.06
R_4C_1	0.9255	0.77	1.3276	1.16	0.9727	1.00
C_2	1.2210	1.05	1.3770	1.20	1.3420	1.38
C_3	1.0995	0.93	1.6085	1.41	1.0970	1.12
R_5C_1	1.0454	0.90	1.2729	1.10	0.9845	1.01
C_2	1.5016	1.29	1.6372	1.45	1.2725	1.31
R_6C_1	1.4634	1.26	1.5253	1.34	1.0808	1.12
Check	0.7007	0.60	0.7926	0.64	0.4576	0.46

produce the required soil-moisture, the total salts per culture in corresponding cultures of the three series were also equal throughout but the total salts per unit volume of soil were directly proportional to the soil-moisture; that is, the total salts added were present in the proportions of 3:6:8 in the corresponding cultures of the sub-optimal, optimal, and supra-optimal series, respectively.

Inspection of the data in table 3 and a comparison of the triangular diagrams in figure 1 show at once that there is a marked degree of similarity between the three series comprised in this group with respect to the positions in the series of the cultures which produced high yields. The areas showing the highest seven yields for each of the three series occupy the same general region at the lower right on the triangular diagrams. Out of the group of cultures in each series which produced the highest seven yields, four are corresponding cultures of the three series and are included in the areas of high yields on each of the three diagrams. These cultures are R_1C_4, R_2C_4, R_2C_5, and R_3C_4. Culture R_1C_3 appears in the area of high yields on the diagrams of the sub-optimal and supra-optimal series but is not included in this area on the diagram of the optimal series. Likewise culture R_2C_3 is included in the area of high yields on the diagram of the optimal and supra-optimal series but is not included in this area on the diagram of the sub-optimal series.

The maximum yield of the sub-optimal series was produced by culture R_1C_4, which, however, did not correspond to the culture giving the highest dry weight in either of the other two series of this group. The maximum yields obtained from the optimal and supra-optimal series were produced by corresponding cultures, each of the cultures having the number R_3C_4 in its respective series. In the optimal series, however, cultures R_1C_5 and R_3C_4 show the same relative value. Each of these cultures is marked with a circle on the diagrams representing the series.

It is thus at once apparent that, under the conditions to which the cultures of this group were subjected, the fertilizer balance for good growth was not materially altered by the rather pronounced differences in the moisture-content of the cultures. This follows from the fact that the areas of high yields on the triangular diagrams representing the three series occupy the same general region at the lower right of the diagrams, four out of the highest seven yields in each series being produced by corresponding cultures. The conclusion is further supported by the fact that the maximum relative yield in the optimal and supra-optimal series was produced by culture R_3C_4 which was also included in the area of high yields in the sub-optimal series, although the maximum relative yield in this series was produced by another culture (R_1C_4).

The average absolute yield-values of the three series taken from the columns of table 3 were arranged in the descending order of the values for the optimal series. These values were then plotted as ordinates to form the graphs of figure 2 in which the culture numbers represent the abscissas.

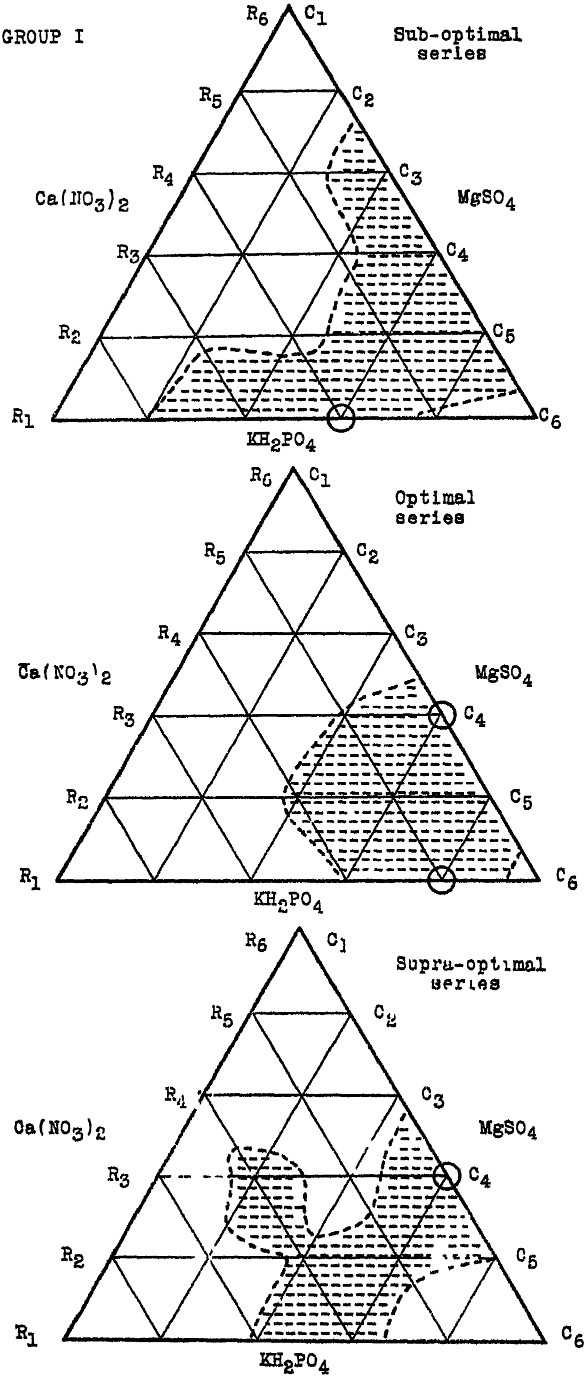


FIG. 1. DISTRIBUTION OF CULTURES PRODUCING THE HIGHEST SEVEN YIELDS OF BUCKWHEAT TOPS IN EACH SERIES OF GROUP I

The cultures giving maximum yields are marked with circles

The three graphs of figure 2 all agree in a general way in showing a gradual downward slope to the right. This indicates, of course, that the proportions of the fertilizer constituents designated by the culture numbers, in passing from left to the right, have a progressively less efficient balance for the growth of buckwheat in the particular soil employed. The graphs representing the sub-optimal and supra-optimal series (30 per cent and 80 per cent soil-moisture, respectively) are, of course, irregular and while there is a tendency toward parallelism between these two graphs in the upper half of their course they are not at all parallel to the graph of the optimal series.

The graph representing the optimal series in which the cultures had a medium soil-moisture content lies considerably above the other two graphs. Not a single culture of this series is surpassed in yield by a corresponding culture of the sub-optimal series and only one by the corresponding culture (R_3C_2) of the supra-optimal. It is to be observed also that the graph representing the supra-optimal series lies above that of the sub-optimal series throughout nearly its entire length, the yields from only three cultures of the former being inferior to the yields from the corresponding cultures of the latter.

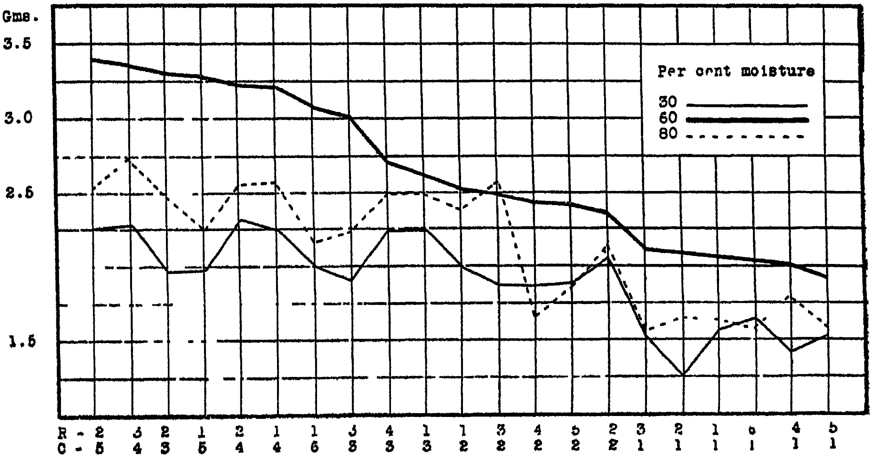


FIG. 2. AVERAGE ACTUAL YIELDS OF BUCKWHEAT TOPS FOR LOW, MEDIUM, AND HIGH SOIL-MOISTURE (SUB-OPTIMAL, OPTIMAL, AND SUPRA-OPTOMAL SERIES, RESPECTIVELY) OF THE CULTURES OF GROUP I

The maximum average absolute yield from the optimal series is 46 per cent higher than that from the sub-optimal and 25 per cent higher than that from the supra-optimal, while the average of the highest seven yields from the optimal series is 45 per cent higher than the corresponding yield from the sup-optimal series and 27 per cent higher than that from the supra-optimal. The superiority of the yields from the cultures of the optimal series over those from the sub-optimal and supra-optimal is thus seen to be quite pronounced.

From the foregoing facts and from the evidence to be presented in connection with the consideration of groups II and III, it is apparent that this superiority of the yields from the cultures of the optimal series is the direct result of more favorable moisture conditions for growth.

In this connection it is to be emphasized that, in general, the influence upon the growth rates, as indicated by yield values, of such differences in soil-moisture content as existed between the cultures of the diffreent series is quite as pronounced as is the influence of the variations in the proportions of the fertilizer constituents as these occur throughout each of the three series.

Group II

In this group, equal amounts of soil were used for all the cultures of the three series. Therefore, the amounts of solution which it was necessary to add to corresponding cultures of the three series in order to produce the required initial moisture-content of the cultures were directly proportional to the initial soil-moisture, as were then also the total salts per culture and the total salts per unit-volume of soil. ,This condition, of course, required that the total concentration of the solutions added to the soil should always be the same.

Comparing now the triangular diagrams of figure 3 which show the distribution of the cultures producing the highest seven yields in each of the three series as shown in table 4, it will be observed that the agreement between the areas marking the positions of these cultures in the different series is even more pronounced than that between the corresponding areas on the diagrams (fig. 1) representing the series of the preceding group. As indicated on the diagrams of figure 3, the areas of high yields all occupy the same general region at the lower right of the triangles. Five cultures of those which produced the highest seven yields in each series are corresponding cultures and appear in the areas of high yields on each diagram. These cultures are R_1C_4, R_2C_3, R_2C_4, R_2C_5, and R_3C_4. It will be observed also that culture R_1C_5 is included in the area of high yields on the diagram of the sub-optimal series and optimal series but not on that of the supra-optimal series, while culture R_1C_6 appears in the shaded area on the diagram of the sub-optimal and supra-optimal series but not on that of the optimal. Similarly culture R_3C_8 is included in the area of high yields on the diagram of the optimal and supra-optimal series but does not appear in this area on the diagram of the sub-optimal.

The maximum yields in the three series of this group were not produced by corresponding cultures although culture R_1C_5 which produced the maximum relative yield in the sub-optimal series corresponds to the culture which produced a secondary maximum yield in the optimal series. The relative yields from two cultures (R_1C_5 and R_3C_4) of this series show equal values. The maximum yield from the supra-optimal series is indicated for culture R_2C_4.

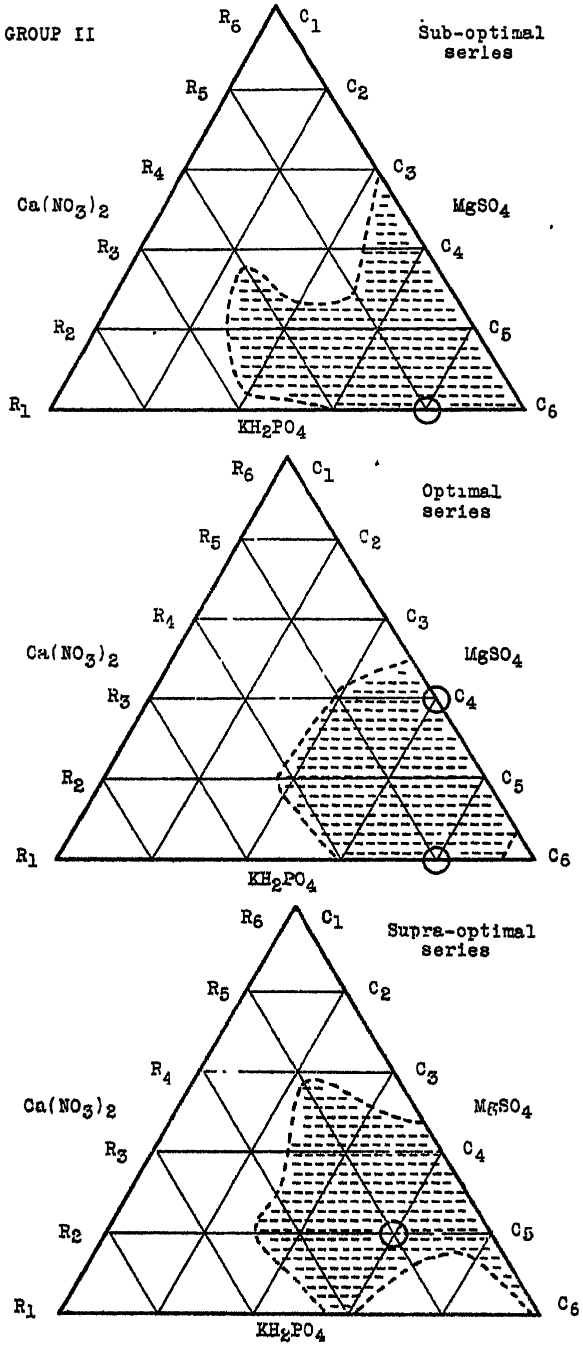


FIG. 3. DISTRIBUTION OF CULTURES PRODUCING THE HIGHEST SEVEN YIELDS OF BUCKWHEAT TOPS IN EACH SERIES OF GROUP II

The cultures giving maximum yields are marked with circles

The diagrams of figure 3, like those of figure 1, show very clearly that there is no significant shifting of the balance of fertilizer constituents characterizing good growth in changing from a soil-moisture content which was considerably below that of the optimum to one above the optimum. This is true for the sets of experimental conditions under which the cultures of this group and those of the preceding group were carried out. A comparison of the diagrams of figure 3 with the corresponding ones of figure 1 shows that these diagrams are in very close agreement with respect to the positions on the diagrams of the areas of high yields, since all occupy somewhat similar regions at the lower right of the triangles. This general agreement is emphasized by the fact that four cultures among those which produced the highest seven yields in each of the six series represented in figures 1 and 3 are corresponding cultures and are included in the areas of high yields on each of the six triangular diagrams. In this connection it is to be noted that in the preparation of the soil cultures of groups I and II the total concentration of the solutions added to the soil to create the required initial soil-moisture was always the same (1.75 atm.) and the total salts per unit volume of soil in corresponding cultures of the three series in each of the two groups were always directly proportional to the soil-moisture content, as shown in table 1. Alteration of these two important factors (since it was not possible to alter the one without altering the other) produced markedly different results with respect to the salt balance required for good growth of buckwheat in the soil used, as will appear in considering the data of group III.

The average absolute yield-values for the three series of group II taken from the columns of table 4 were plotted to form the graphs shown in figure 4. These graphs were prepared in precisely the same manner as were those (fig. 2) representing the corresponding series in the preceding group. It will be observed that the arrangement of the graphs of figure 4 is quite similar to that of the graphs of figure 2. The graph representing the yields from the optimal series with medium soil moisture is again seen to lie considerably above the other two graphs. Thus under the cultural conditions defined at the beginning of this section and expressed numerically in table 1, every culture of the optimal series except one (culture R_1C_1) shows a marked superiority in yield over the corresponding cultures of the other two series in spite of the fact that the cultures of the supra-optimal series received a total salt application per culture and per unit volume of soil which was considerably higher than that received by the cultures of the optimal series. On the other hand, the cultures of the sub-optimal series received a total salt application which was only half as great as that received by the cultures of the optimal series. However, when the cultures of the sub-optimal and of the supra-optimal series received total salt applications which equaled those received by the cultures of the optimal series the relation between this series and the other two, with respect to the yields produced, was not materially altered as is clearly shown by a comparison of the graphs of figures 2, 4, and 6.

The superiority of the yields from the optimal series over those from the other two series is emphasized by the fact that the maximum yield from this series was 63 per cent above that of the sub-optimal and 11 per cent higher than that of the supra-optimal, while the average of the highest seven yields from the optimal series was 69 per cent and 25 per cent higher than the corresponding yields of the sub-optimal and supra-optimal series, respectively.

The graph representing the supra-optimal series (fig. 4) lies considerably above that of the sub-optimal throughout almost its entire length, only a single culture (R_1C_5) of the former series being surpassed in yield by the corresponding culture of the latter. Thus the relation between these two graphs is quite similar to that exhibited by the graphs representing the corresponding series of the preceding group (fig. 2).

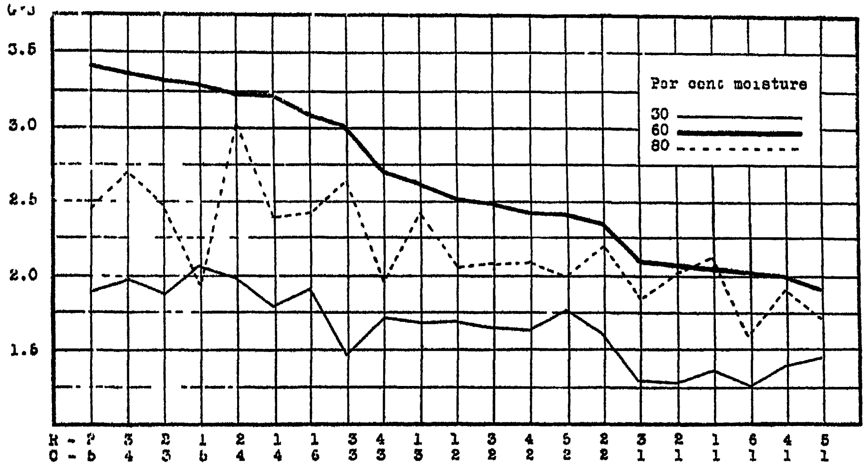


FIG. 4. AVERAGE ACTUAL YIELDS OF BUCKWHEAT TOPS FOR LOW, MEDIUM, AND HIGH SOIL-MOISTURE (SUB-OPTIMAL, OPTIMAL, AND SUPRA-OPTIMAL SERIES, RESPECTIVELY) OF THE CULTURES OF GROUP II

Group III

In this group, as in group II, equal amounts of soil were used in all the cultures and the amounts of solution added to the soil cultures of the three series in order to produce the required initial soil-moisture were of necessity again made directly proportional to the moisture-content of the cultures. The proportions were as 3:6:8 in the cultures of the sub-optimal, optimal, and supra-optimal series, respectively. Although the salt proportions of the solutions added to corresponding cultures were always the same it is important to note that the total salt concentrations of the solutions differed widely since they were inversely proportional to the soil-moisture content. The solutions added to the cultures of the sub-optimal, optimal, and supra-optimal series had total osmotic concentration values of 3.5. 1.75, and 1.31 atmospheres,

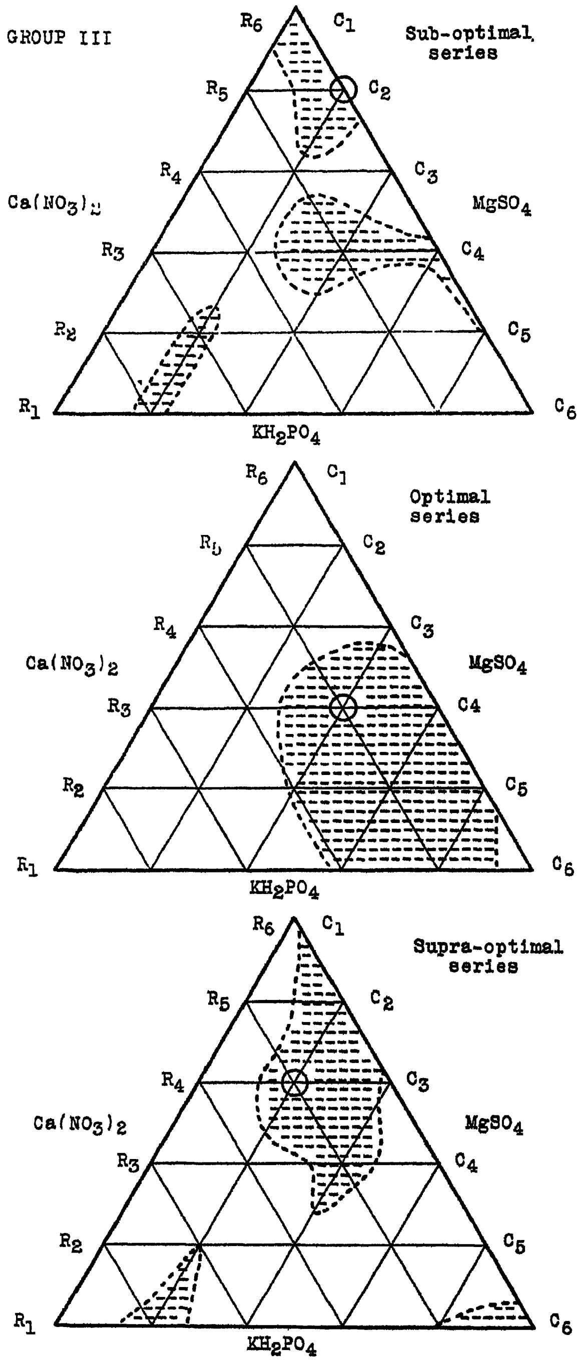


FIG. 5. DISTRIBUTION OF CULTURES PRODUCING THE HIGHEST SEVEN YIELDS OF BUCKWHEAT TOPS IN EACH SERIES OF GROUP III

The cultures giving maximum yields are marked with circles

respectively. The total salts per culture and the total salts per unit volume of soil in corresponding cultures were, therefore, equal throughout as indicated by the relative data in table 1.

The triangular diagrams graphically representing the three series of this group are shown in figure 5. It will be observed that there is scarcely any agreement, with respect to the areas marking high yields, between the diagrams of the optimal series and those of the other two series. On the diagrams of the sub-optimal and supra-optimal series the high yields are included in small detached areas nor do these occupy the same general region at the lower right on the diagrams as do the areas of high yields of all the series comprised in the preceding groups (figures 1 and 3) and that of the optimal series in the present group. Only a single culture (R_3C_3) of those which produced the highest seven yields in the respective series appears in the area marking these on the diagram of each of the three series. However, out of the highest seven yields from the sub-optimal series and from the supra-optimal series four were produced by corresponding cultures and these are shown in the detached areas of high yields on the diagrams representing these two series, but there is little similarity between the areas of high yields on the two diagrams. The main point of agreement lies in the small area at the lower left on each triangle embracing the two cultures R_1C_2 and R_2C_2.

In the sub-optimal series the maximum relative dry weight yield was produced by culture R_5C_2, in the optimal series by culture R_3C_3, and in the supra-optimal series by culture R_1C_2.

It is thus evident that the fertilizer balance characterizing the cultures which produced high yields in all the series of the preceding groups and in the optimal series of the present group is markedly different from that characterizing the high yielding cultures in either the sub-optimal or the supra-optimal series of this group. This alteration of the salt balance characterizing high yields in the sub-optimal and supra-optimal series may be attributed directly to the difference in the total concentration of the solutions added to the cultures of the different series to produce the required initial soil moisture. The solutions added to the cultures of the sub-optimal, optimal, and supra-optimal series had total osmotic concentration values of approximately 3.5 1.75, and 1.31 atmospheres, respectively, but the total salts per culture as well as the total salts per unit volume of soil were equal throughout. That this difference in the fertilizer balance required for good growth is not due to the variations in the moisture-content of the cultures of the different series is clearly brought out by the fact that, as shown in table 1, the solutions added to the cultures of the series in groups I and II, as well as to those in the optimal series of group III, all had approximately the same total osmotic concentration value (1.75 atm.), and that in the series in question, seven in all, no significant alteration in the salt balance characterizing the cultures which produced high yields was observed with change from the highest to the lowest soil-moisture content. Pronounced shifting of the balance of salt proportions characterizing good growth in the soil here used occurred only with notable differences in the total concentrations of the solutions employed to produce the required initial soil-moisture, and such differences in total salt concentration existed only in the solutions added to the cultures of the sub-optimal and supra-optimal series of group III, the triangular diagrams of which are shown in figure 5. In this connection it has been pointed out by Gile (3), McCool (8, p. 113–170), Tottingham (11) Shive (9), McCall (7), Ayres (1), and others that the balance of salt proportions required for plant growth (good, medium, or poor) is not at all independent of the total salt concentration of the medium in which the plants are rooted, a conclusion which is supported by the present work.

The absolute average dry-weight yields from the series of group III, as given in table 5, are presented graphically in figure 6 in the same manner as were those from the series of the preceding groups. The graph of the optimal series again lies above that of the supra-optimal throughout but its lowest two values are slightly surpassed by the corresponding values of the sub-optimal graph. With the exception of the two yield-values just mentioned, the cultures of the optimal series exhibit much higher average dry weights than do those of the other two series. This relation is quite similar to that shown to exist between the corresponding series of the preceding groups.

The maximum yield from the optimal series was 33 per cent higher than that from the sub-optimal and 49 per cent above that of the supra-optimal. The average of the highest seven yields from the optimal series was 22 per cent and 45 per cent higher than the corresponding yields from the sub-optimal and supra-optimal series, respectively.

Thus in the present group, as in the preceding ones, the differences in yield resulting from differences of soil-moisture in corresponding cultures are even more pronounced than are differences in yield which may be attributed to variations in the proportions of the fertilizer constituents throughout each series. This emphasizes the point to which attention was given in the earlier publication (10) that in all soil or sand culture studies it is of primary importance to maintain the moisture-conditions of the substratum in which the plants are grown within very narrow variation limits where the influence of other factors under investigation, such as the kind, total concentration, and proportions of fertilizer constituents, is being measured by the growth rates of the plants.

The graph of the sub-optimal series lies above that of the supra-optimal series throughout most of its length. Only three values of the former are surpassed by the corresponding values of the latter. The relation between these two graphs is, therefore, the reverse of that exhibited by the graphs of the corresponding series in both groups I and II (figures 2 and 4). This reversed relation between the graphs in question indicates that, in pot-cultures such as were here used, with any given set of fertilizer constituents, the total salts per unit-volume of soil in which the plants are grown bear a more important relation to the growth rates of the plants than do the total salts per culture. This appears from the fact that in the sub-optimal and supra-optimal series of group I the total salts per culture were present in corresponding cultures in the ratio of 1:1, while in group II this ratio was 1.00:2.66, yet the relation between the graphs representing the two series in each group are quite similar as a comparison of the graphs in figures 2 and 4 will show. Nor did differences in the amounts of soil used per culture appear to have any influence upon this relation. On the other hand, the total salts per unit-volume of soil in corresponding cultures of the sub-optimal and supra-optimal series in both groups I and II were present in the ratio of 1.00:2.66, while in group III this ratio was 1:1. Thus, with the change in the total salts per unit-volume of soil in corresponding cultures, the relation between the graphs representing the two series suffered a complete reversal, as is shown by a comparison of the graphs in figure 6 with those in figures 2 and 4, while a like change in the total salts per culture had no apparent influence upon the relation between the graphs representing the two series. It is to be observed, of course, that any alteration in the ratio of total salts per unit-volume of soil in corresponding cultures of the different series above noted involved also a corresponding change in the total concentrations of the solutions with which the initial salt applications were supplied to the soil.

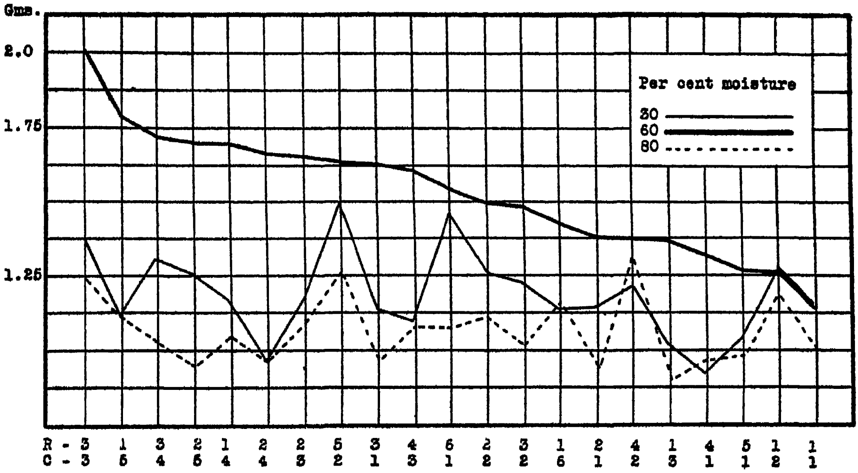


FIG. 6. AVERAGE ACTUAL YIELDS OF BUCKWHEAT TOPS, FOR LOW, MEDIUM, AND HIGH SOIL-MOISTURE (SUB-OPTIMAL, OPTIMAL, AND SUPRA-OPTIMAL SERIES, RESPECTIVELY) OF THE CULTURES OF GROUP III

A comparison of the three sets of graphs (figures 2, 4, and 6) representing the average actual yields shows that the highest seven yields from the series in which the cultures had an approximately optimum soil-moisture, were always greatly superior to the corresponding yields from the series in which the cultures had a soil-moisture content which was either above or below that of the optimum, regardless of the different experimental conditions to which the cultures were subjected. This relation was not altered by the use of larger or smaller amounts of soil per culture than that used for the cultures of the optimal series, by higher or lower total concentrations of the solutions added to the soil-cultures to create the required initial moisture-content, nor by higher or lower salt applications per culture or per unit-volume of soil. It is thus to be expected that any fertilizer mixture in which the salt proportions are properly balanced for the good growth of a given species in a given soil can not be most efficiently utilized by the plants if the moisture-content of the substratum is either too high or too low, nor can fertilizer treatment alone entirely counteract the retarding influence upon plant growth of unfavorable moisture-conditions. The maximum plant-producing value of any fertilizer mixture can only be attained when the moisture-condition of the substratum is at the optimum for plant growth.

From the experimental point of view, the importance of considering moisture relations in solid substrata in connection with plant-culture studies can not be too strongly emphasized. This applies as well to field studies dealing with the influence of soil treatment upon the growth rates of plants and upon crop production. The fact that soil-moisture in the field is quite variable and exceedingly difficult to control does not render it less important as a factor to be considered in the interpretation of experimental data. As Harris (4) has pointed out, soil-moisture has always been a troublesome factor in experiments with soils and crops and the effect of unavoidable differences in moisture-conditions frequently obscures the influence of soil treatment and renders entirely worthless data which have been obtained at the expense of much time and labor.

SUMMARY

A study was made of the effect of different degrees of moisture upon physiological salt balance as this affected the growth of buckwheat plants in soil cultures. Three different degrees of soil-moisture were maintained: 30 per cent, 60 per cent, and 80 per cent based on the maximum water-retaining capacity of the soil used. Tests were made with 21 different sets of salt proportions of the three salts KH_2PO_4, $Ca(NO_3)_2$, and $MgSO_4$ supplied to the air-dry soil in the solution form. The solutions were added to the soil in the proper amounts to create the required initial soil-moisture. The methods by which this was accomplished are described. Nine series, each comprising 21 cultures, divided into three groups of three series each were conducted simultaneously and repeated. The cultures were weighed and the water loss by transpiration was restored daily throughout the growth period. A brief summary of the main results derived from the experimental data follows:

1. The balance of salt proportions which characterized the cultures producing high yields was not materially altered by variations in the degrees of soil-moisture when the total osmotic concentration values of the solutions added to the soil cultures to produce the required initial soil-moisture were

approximately constant. In general the salt proportions which produced high yields of tops with the lowest degree of soil-moisture gave high yields also with the medium and the highest degree of moisture in the respective series.

2. Pronounced shifting of the balance of salt proportions characterizing the cultures which produced high yields occurred only with marked variations in the total concentration of the solutions added to the soil.

3. The yield-differences resulting from variations in the degrees of soil-moisture are quite as pronounced as are yield differences which may be attributed to variations in the proportions of the fertilizer constituents with constant soil-moisture.

4. The retarding influence of unfavorable soil-moisture conditions upon the rate of growth of the plants can not be counteracted by fertilizer treatment alone. High yields from cultures with medium soil-moisture (60 per cent on the basis of maximum waterholding capacity of the soil) were always greatly superior to the corresponding yields from the cultures with either high or low soil-moisture regardless of experimental methods or conditions to which the cultures were subjected.

5. The salts comprised in a fertilizer mixture can not be efficiently utilized by the plants under unfavorable moisture-conditions. The maximum plant-producing value of any fertilizer mixture can only be attained when the moisture-conditions of the substratum are at the optimum for plant growth.

REFERENCES

(1) Ayres, A. H. 1917 Influence of the composition and concentration of the nutrient solution on plants grown in sand cultures. *In* Univ. Cal. Pub. Agr. Sci., v. 1, no. 11, p. 341–394.

(2) Briggs, L. J., and Schantz, H. L. 1911 A wax seal method for determining the lower limit of available soil moisture. *In* Bot. Gaz., v. 51 no. 3, p. 210–219

(3) Gile, P. L. 1913 Lime-magnesia ratio as influenced by concentration. Porto Rico Agr., Exp. Sta. Bull. 12.

(4) Harris, F. S. 1914 Effect of variations in moisture content on certain properties of a soil and on the growth of wheat. N. Y. (Cornell) Agr. Exp. Sta. Bull. 352.

(5) Hilgard, W. E. 1918 Soils, their formation, properties and composition. MacMillan, New York.

(6) Livingston, B. E. ed. 1919 A Plan for Cooperative Research on the Salt Requirements of Representative Agricultural Plants. The Johns Hopkins University, Baltimore.

(7) McCall, A. G. 1916 The physiological balance of nutrient solutions for plants in sand cultures. *In* Soil Sci., v. 2, no. 3, p. 207–253.

(8) McCool, M. M. 1913 The antitoxic action of certain nutrient and non-nutrient bases with respect to plants. *In* N. Y. (Cornell) Agr. Exp. Sta. Mem. 2.

(9) Shive, J. W. 1915 A study of physiological balance in nutrient media. *In* Physiol. Res., v. 1, no. 7, p. 327–397.

(10) Shive, J. W. 1920 Relation of moisture in solid substrata to physiological salt balance for plants and to the relative plant producing value of various salt proportions. *In* Jour. Agr. Res., v. 18, no. 7., p. 357–378.

(11) Tottingham, W. E. 1914 A quantitative chemical and physiological study of nutrient solutions for plant cultures. *In* Physiol. Res., v. 1, no. 4, p. 133–245.

ERRATA

p. 28, lines 16–18 should read:

Synthetic acid medium: 1000 cc. distilled water, 10 gm. dextrose, 5 gm. peptone, 1 gm. KH_2PO_4, 0.5 gm. $MgSO_4$, add acid to adjust pH to 4.0, 25 gm. agar.

p. 101, lines 11–12 should read:

(10) MELLOR, J. W. 1913 Higher Mathematics for Students of Chemistry and Physics. Longmans, New York.

p. 132, line 23, the following table should be inserted:

TABLE 4

Fluctuation of nitrogen in samples isolated in May, incubated in pots, and analyzed after monthly intervals

TIME OF ANALYSIS	INCREASE IN NITROGEN CONTENT			
	Light soil (initial N content was 36.1 mgm. per 100 gm.)		Heavy soil (initial N content was 37.5 mgm. per 100 gm.)	
	per cent	*per cent*	*per cent*	*per cent*
June	9.4	5.8	9.4	7.7
July	−4.1	−20.6	0.5	−8.3
August	−6.1	0.0	3.5	1.1
September	6.9	−1.1	8.3	4.8
October	10.0	10.2	5.1	6.9

p. 217, footnote should read:

[1] Paper No. 82 of the Journal Series, New Jersey Agricultural Experiment Stations, etc.

THE EFFECT OF NODULE BACTERIA ON THE YIELD AND NITROGEN CONTENT OF CANNING PEAS[1]

E. B. FRED AND O. C. BRYAN

University of Wisconsin

Received for publication January 11, 1922

INTRODUCTION

The study of the actual amount of nitrogen which inoculated legumes take from the air has almost invariably led to different results. These differences can be expected because of the variation in soil types and because of the nature of the different leguminous plants. A general discussion of the nitrogen sources of legumes is out of place here; the reader is referred to a report by Brown and Stallings (1). It may be pointed out, however, that according to the generally accepted statement, inoculated legumes increase the nitrogen supply of the soil. Accurate data supporting this statement and showing the amount of nitrogen which legumes add to the soil have been determined for certain soils and certain leguminous plants, but unfortunately little of this information has been obtained under field conditions. Because of the importance of combined nitrogen for soil fertility, it is desirable to measure carefully, under field conditions, the influence of legumes on the supply of nitrogenous compounds in the soil.

PROCEDURE AND RESULTS OF THIS STUDY

In a former paper (2) some results of field tests with Alaska peas, inoculated and uninoculated, on poor Plainfield sand and on rich Carrington silt loam soil were described. It was noted here that inoculation brought about an increase in the total yield of pea plants, in the yield of peas, and in the percentage of nitrogen in the peas.

In this paper, the influence of nodule bacteria on the yield of canning peas, and on the amount of nitrogen taken from the air will be considered. In the spring of 1921 two different types of Miami silt loam soils were selected for study. They were both slightly acid and had not been planted to peas for several years. One of the soils had been cultivated for only three years, contained 0.228 per cent of total nitrogen and was in a good state of fertility. The other soil had been cropped in grain and corn for the last five years and the total nitrogen in this soil was 0.180 per cent. Identical experiments, in so far as it was possible, were carried out on both of these soils. The plots were laid out twelve feet wide on a uniform section of field and extended across the entire length of field. The peas were planted with a grain drill. The checks were planted first. All inoculations were made with pure cultures of bacteria. The same variety of peas, *Horsford*, was grown on both soils and was planted at the rate of 4 bushels per acre.

---

[1] Published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

On May 25, about 30 days after the time of seeding, the plants were examined, but no difference in the size of the plants from the different plots was evident. At this time the roots of the inoculated plants on both soil types had numerous nodules while the uninoculated showed only a few. The effect of inoculation was already noticeable in the darker green color of these plants.

When the majority of the peas had reached the proper stage for canning, representative parts of the inoculated and uninoculated plots on the more fertile soil, $\frac{1}{500}$ of an acre each, were measured off. The plants were carefully harvested, counted, and weighed. These data as well as the nitrogen contents are given in table 1. Comparison of weights shows that in spite of the decrease[2] in the number of plants, there were more pounds of tops per acre on the inoculated than on the uninoculated plot. It is true that no gain in weight of pods and peas could be noted and yet, on analysis, a substantial increase in the percentage of nitrogen was seen. If calculated as pounds per acre, the increase in yield above that of the control amounts to 217.4 pound of dry tissue and a gain of 37.25 pound of nitrogen. Since some of the plants in the uninoculated plot became infected this difference in nitrogen between the inoculated and uninoculated is below the actual amount of nitrogen fixed from the air.

When the peas were ready to can representative parts of the inoculated and uninoculated plots on the less fertile soil, $\frac{1}{500}$ of an acre each, were measured off and the entire crop was harvested. The plants were dug up carefully; the roots and nodules were freed from the soil. Because of its open texture, the soil could be easily removed from the roots without a great loss of nodules. The nodules were picked from the roots and analyzed. The results of the analysis of the tops and roots, peas and hulls, and the nodules are given in table 2.

Although not great, there was a well defined difference between the inoculated and uninoculated plants. The peas treated with nodule bacteria were somewhat taller and of a darker green color. The total yield of peas was only about one-half that obtained from the more fertile soil. At best, the yield of peas on this soil was not more than half of a normal crop.

Here, as in the previous test, there are fewer plants on the inoculated section than on the uninoculated. The effect of bacteria on the yield and on the nitrogen content of the plants is readily seen from the figures in this table. With the exception of the seed, there is a well defined gain in yield of the inoculated plants as compared with the uninoculated. It is in the percentage of nitrogen in the different parts of the plant that the result of inoculation becomes most noticeable; there is a gain in the tops and roots, in the hulls, and also in the peas. Although almost without effect on the yield of crop, the nodule bacteria brought about a substantial increase in the percentage of nitrogen.

---

[2] The decrease in number of plants in the inoculated plots is no doubt due to the number of seed planted. Treatment of the pea seed with a water suspension of the nodule bacteria caused them to swell. Similar results were obtained in a second test.

For the each acre treated, the treated plants show a net gain of 179.0 pounds of tops and roots and 51.5 pounds of hulls. The gain in nitrogen of the inoculated crop over that of the uninoculated crop was 13.2 pounds.

TABLE 1

The yield and amount of nitrogen in canning peas grown with and without inoculation on Miami silt loam soil

TREATMENT	NUMBER OF PLANTS	TOTAL YIELD (DRY)	DRY WEIGHT		NITROGEN	
			Tops	Peas and pods	Tops	Peas and pods
		gm.	*gm.*	*gm.*	*per cent*	*per cent*
Inoculated	575	2055	1715	340	3 67	4.42
Uninoculated	711	1858	1518	340	2.17	3.31
Gain due to inoculation	−136	+197	+197	0	+1.50	+1.11

TABLE 2

The yield and amount of nitrogen in inoculated and uninoculated canning peas on poor Miami silt loam soil

TREATMENT	NUMBER OF PLANTS	YIELD (DRY WEIGHT)					NITROGEN			
		Total	Tops and roots	Hulls	Peas	Nodules	Tops and roots	Hulls	Peas	Nodules
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*	*per cent*	*per cent*
Inoculated	545	1480	1162	210	94	14.0	1.97	2.34	4.46	1 49
Uninoculated	572	1256	1000	163	93	0.0	1.44	1.61	3.50	0.0*
Gain due to inoculation	−27	+224	+162	+47	+1	+14.0	+0.53	+0.73	+0.96	+1.49

* A few nodules present but not enough for analysis.

This beneficial effect of inoculation on garden peas, which is noticeable not only in the total nitrogen but also in the yield, is probably much greater than the figures indicate. As is the case with nearly all field tests, the uninoculated plants were not entirely without nodules and accordingly were benefiting from the bacteria naturally present in the soil.

REFERENCES

(1) Brown, P. E., and Stallings, J. H. 1921 Inoculated legumes as nitrogenous fertilizers. *In* Soil Sci., vol. 12, p. 365–407.

(2) Fred, E. B., Wright, W. H., and Frazier, W. C. 1921 Field tests on the inoculation of canning peas. *In* Soil Sci., vol. 11, p. 479–485.

THE FORMATION OF NODULES BY DIFFERENT VARIETIES OF SOYBEANS[1]

E. B. FRED AND O. C. BRYAN
University of Wisconsin

Received for publication July 10, 1922

Variation in nodule formation among different varieties of soybeans has been reported from different parts of Wisconsin. In many cases a certain variety has failed to show inoculation when treated with a culture of known origin, while among different varieties growing side by side one has been inoculated and the other has remained free or practically free from nodules. Similar observations have been made in other parts of the United States.

Vorhees (2) of New Jersey found that in a mixture of Brown and Haberlandt grown on medium heavy clay loam soil the two varieties did not show the same nodule formation. Although their root systems were closely associated in the soil, only the Brown variety produced nodules. Morse (2), in discussing Voorhees' paper, reports that at the West Tennessee Experiment Station the Acme and Tokio varieties of soybeans failed to form nodules, while the Mammoth variety under the same conditions formed many nodules. Similar results were obtained the next year.

Leonard (1), on the other hand, as a result of laboratory and green-house tests concluded that a culture of bacteria isolated from a single strain of soybeans will form nodules on other varieties. In his experiments the organism was isolated from the Medium Yellow variety of soybean. Nineteen different varieties of soybeans were studied, including Amherst, Arlington, Barchet, Chernie, Chestnut, Cloud, Guelph, Haberlandt, Hope, Ito San, Jet Manhattan, Medium Yellow, Pekin, Taha, Virginia and Wilson.

It seems from these reports that the ability to form nodules varies among the different kinds of soybeans. Apparently certain varieties are more easily inoculated than others. It is also true that the time of ripening of one variety may influence the infection and subsequent formation of nodules. For instance, an early and late variety may show differences in inoculation as a result of variation in plant food at different times of the year. Repeated investigations have proved that the formation of nodules is influenced to a considerable degree by the reaction and salt concentration of the soil water as well as by other factors.

Because of the importance of soybeans in Wisconsin, it seemed of interest to measure under field and laboratory conditions the effect of different strains of soybean bacteria on the assimilation of nitrogen by different varieties of soybean plants and to contrast pure-culture inoculation with soil inoculation. The results of these studies are presented in this paper.

---

[1] Published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

Pure cultures of the different strains of bacteria were obtained from known varieties of soybeans, and their ability to cause inoculation was determined previous to these experiments. The soil used for inoculation was from a field on the Experiment Station Farm where well inoculated soybeans had been grown for several years. Although the records are not available, it is highly probable that the varieties grown on this soil were Wisconsin Black and Ito San soybeans.

GREENHOUSE TESTS

Glazed earthenware pots were filled with pure white silica sand to which had been added an excess of $CaCO_3$. These were sterilized and then planted in duplicate for each culture to Manchu, Mammoth Yellow, Wisconsin Black, and Medium Early Green soybeans, which had been treated immediately preceding planting with either the desired pure culture or with soil. For example, Manchu soybeans were inoculated with bacteria from Mammoth Yellow, Medium Yellow, Haberlandt, and Manchu varieties of soybean. Nitrogen-free plant food was added at intervals in quantities sufficient for plant growth.

TABLE 1

Effect of different strains of soybean bacteria on the nitrogen content of different varieties of soybeans, greenhouse test

SOURCE OF BACTERIA	NITROGEN CONTENT OF TOPS, ROOTS AND NODULES			
	Manchu	Mammoth Yellow	Wisconsin Black	Medium Early Green
	per cent	*per cent*	*per cent*	*per cent*
Not inoculated	1.85	1.64	1.35	1.42
Mammoth yellow	2.85	3.13	2.95	
Medium yellow	3.02	2.95	2.74	
Manchu	3.02	3.02	2.68	
Haberlandt	3.01	2.85	2.76	2.93
Soil from soybean field	2.37	2.79	2.45	2.87

Within three weeks after planting, a well defined contrast was noticeable in size and color of the inoculated and uninoculated plants. The uninoculated plants were smaller and at the end of five to six weeks decidedly yellow. At this stage the plants were washed from the sand and their appearance as to size, color, and inoculation was carefully noted.

The combined tissue of the two parallel pots was used for analysis. Table 1 gives the percentage of nitrogen in the dry soybean plants, tops, roots, and nodules. Under the conditions of this experiment all indications are that pure cultures of bacteria from the nodules of Mammoth Yellow, Manchu, Haberlandt, or Medium Yellow soybeans may be used interchangeably on the different varieties of the soybeans and are almost equally effective in assimilating nitrogen.

The same conclusions are to be drawn from the use of well inoculated soil as a source for soybean bacteria. The general appearance of the soil inoculated plants as to size and color of plants and number of nodules was equally as good as that of the plants inoculated with pure cultures, and on analysis the tissue showed a comparable percentage of nitrogen fixed.

FIELD TESTS

A plot of sandy soil on the farm of Mr. J. R. Williams at Montello was selected for this study. This soil had never grown soybeans. It had been cropped in corn and grain for the last 6 or 8 years and was in rather poor state of fertility. No fertilizer or manure was added. The reaction was medium acid. On May 25 the plots were laid out; each plot consisted of two 30-inch rows twelve rods long, planted to soybeans with a small hand drill. All check plots were planted first; Ito San, Manchu, Mammoth Yellow, Wisconsin Black, Black Eyebrow, and Hollybrook varieties of soybeans were used. Then quantities of each variety of seed sufficient to plant a single plot were inoculated with the Manchu strain of soybean bacteria. When these had been planted the hopper of the planter was washed with water and the operation repeated for the seed which was inoculated with the Mammoth Yellow and the Haberlandt strain of bacteria respectively. On September 27, one-thousandth of an acre was harvested from each plot, bagged, and expressed to Madison. There were practically no nodules on any plants in the check plots, while all plants in the treated plots showed numerous nodules. The plants in the treated plots were in all cases larger and greener than those of the checks.

TABLE 2

Effect of different strains of soybean bacteria on the dry weight and percentage of nitrogen of different varieties in soybean field test

SOURCE OF BACTERIA	WEIGHT AND NITROGEN CONTENT OF DRY STALKS AND PODS							
	Manchu		Wisconsin Black		Ito San		Black Eye-brow	
	lbs.	*per cent*	*lbs.*	*per cent*	*lbs.*	*per cent*	*lbs.*	*per cent*
Not inoculated	306.5	5.45	613.0	6.04	540.2		824.7	5.44
Manchu	573.3	5.36	949.0	6.10	637.2	6.0	1217.2	6.23
Mammoth Yellow	641.7	5.45	826.9	6.45	692.4		1226.0	6.45
Haberlandt	793.8	5.40	959.2	6.48	597.6	5.96	1226.0	6.61

The oven-dry weights of the pods and stalks together with the percentage of nitrogen are recorded in table 2. The Mammoth Yellow and Haberlandt varieties were too late in ripening to be of any value and were not analyzed.

The general appearances were about the same for all varieties of soybeans regardless of the source of the culture of bacteria used to inoculate. Apparently one strain of bacteria was as efficient as another. The uninoculated soybeans produced a much smaller amount of dry seed and also a lower percentage of nitrogen.

From the results of these laboratory and field studies, there is no evidence to conclude that the nodule bacteria of soybeans are highly specific, but on the contrary it was found that the bacteria of one variety will readily infect another. Variation in nodule formation as seen under field conditions must be due to some factor other than difference in bacteria.

REFERENCES

(1) LEONARD, L. T. 1916. Variation in nodule formation. *In* Jour. Amer. Soc. Agron., v., 8, p. 116–118.

(2) VOORHEES, J. H. 1915. Variations in soybean inoculation. *In* Jour. Amer. Soc. Agron., v. 7, p. 139–140.

SULFUR AS AN IMPORTANT FERTILITY ELEMENT[1]

C. O. SWANSON AND W. L. LATSHAW[2]

Kansas Agricultural Experiment Station

Received for publication July 1, 1922

Sulfur is one of the essential elements of plant growth. An element is essential either because it furnishes building material or performs some important physiological function, or both. Sulfur is one of the elements of protein compounds and because of the intimate connection of these compounds with life processes it probably performs physiological functions in connection with the formation of compounds that do not contain sulfur. It follows therefore that profitable crop production is not possible in the absence of an adequate supply of available sulfur any more than it is possible in the absence of an adequate supply of available nitrogen, phosphorus or potassium.

The possibility of sulfur being a limiting element in crop production has only lately been recognized. References to the literature may be found in papers by Lipman and McLean (4), McLean (5), and Ames and Boltz (1). Delay of this recognition was due mainly to incorrect methods of analyses of sulfur both in crops and in the soil. Hilgard (3) gives the following averages for percentages of sulfur in soil: Humid region of the United States, 0.02, transition region, 0.008; arid region, 0.02. These figures of Hilgard are based on the old method for sulfur determination. Shedd (7) found an average of 0.02 per cent sulfur in eight Kentucky soils. Ames and Boltz (1) found that soils in Ohio averaged from 330 to 1112 pounds sulfur per acre surface soil six inches deep. Reimer and Tartar (6) found 0.038, 0.032, 0.02, 0.038, 0.021, 0.027, 0.037, 0.028, 0.029, 0.015, 0.024 and 0.036 per cent of sulfur in Oregon soils. These percentages from the several states are not so widely different from the results obtained on Kansas soils.

The differences in results obtained on soils by the older methods are not as great as those obtained on plant substances. This is shown by the following comparison of analyses by Hart and Peterson (2) with the earlier analyses made by Wolff (10).

The greatest error caused by the old methods of analysis was due to the fact that most of the sulfur was thought to be present in the leaves and stems of the plant which are returned largely to the soil in one form or another, and a comparatively small amount in the seeds, the portion of the plant usually sold from the farm. The amount of sulfur removed from the soil by selling grain according to the old methods of analysis was so small that the loss was more than made up by the addition of sulfur in rain. The recent methods of analysis show that the total sulfur removed in grain is more than that present in the stalks of such crops as corn and wheat.

---

[1] Contribution No. 99 from the Department of Chemistry of the Kansas State Agricultural Experiment Station.

[2] A preliminary report of this investigation was read at the spring meeting of American Chemical Society, St. Louis, 1920. Released for publication in SOIL SCIENCE by courtesy of H. E. Howe, Editor of the *Journal of Industrial and Engineering Chemistry.*

Another reason why the importance of sulfur as a possible limiting element of crop production was not earlier recognized is that sulfur is added to the soil in several of the carriers of the element present in commercial fertilizers. Ammonium sulfate, potassium sulfate and acid phosphate are well known examples of such carriers of sulfur. The calcium sulfate present in a ton of ordinary 16-per cent acid phosphate contains about 200 pounds of sulfur. One hundred pounds of such acid phosphate would then contain approximately as much sulfur as is present in the grain and straw of a 50-bushel wheat crop. Acid phosphate is one of the most important ingredients in commercial fertilizers. Ammonium sulfate and potassium sulfate are also important. Gypsum as land plaster has long been used as a soil amendment. It is thus easy to see that sulfur has been incidentally applied to worn soils in connection with the use of nitrogen, phosphorus and potassium.

TABLE 1

Sulfur content of common farm crops

CROP	SULFUR IN PLANT (HART AND PETERSON)	SULFUR IN PLANT (WOLFF)
	per cent	*per cent*
Alfalfa hay	0.287	0.170
Red clover	0.164	0.089
Maize, grain	0.170	0.004
Maize, stover	0.126	0.113
Oats, grain	0.189	0.002
Oats, straw	0.195	0.092
Timothy	0.190	0.078
Wheat, grain	0.170	0.003
Wheat, straw	0.119	0.053

In a previous investigation at the Kansas State Agricultural College (8) a study was made of the sulfur content of virgin soils in comparison with soils that had been cropped to grain. All the soils, with one exception, included in that study were from the eastern or humid part of the state and almost all were upland soils.

With the exception of two pairs, the soils then studied were not from contiguous fields. The average sulfur content of the cropped fields, compared with the average sulfur content of the fields in virgin sod showed that the cropped soils had 40 per cent less sulfur than the soils in virgin sod. By the same comparison it was shown that the loss of carbon and nitrogen had been 38 per cent for each. The average difference in phosphorus content of the virgin soil compared with the cropped soil was within the experimental error of phosphorus determination consequently it could not be stated that there had been a loss of phosphorus.

These facts warranted further investigation. In conducting the investigation on the effect of alfalfa on the fertility elements of the soil in comparison with grain crops (9), soil samples were collected from 96 fields located in different parts of Kansas. These samples furnished excellent material for the further study of the sulfur problem. The plan followed in collecting these samples was to locate, in different parts of the state, alfalfa fields which had been in this crop for a long time, most of them from 20 to 30 years. Near these alfalfa fields were usually found soils of the same type which had been cropped to grain continuously since broken. As a rule this time was about 40 years. Near these fields cropped to grain or alfalfa, were nearly always found soils in virgin sod. In the central and western part of the state nearly all the fields (alfalfa, grain and sod) were on bottom land. The soil samples were taken in four strata: 0–7 inches, 7–20 inches, 20–40 inches, and 40–80 inches. For the work on sulfur only the surface soils were used.

Sulfur was determined in these soil samples by two methods: The Osborne method using sodium peroxide to oxidize the sulfur; and by a method devised in this laboratory, substituting magnesium nitrate for sodium peroxide. The sodium peroxide method gives good results in the hands of an experienced analyst, but it has a number of disadvantages well known to all who have used the method. The use of magnesium nitrate instead of sodium peroxide has several distinct advantages. The method used as is follows:

The magnesium nitrate solution is made by dissolving 320 gm. of calcined magnesia in nitric acid. The magnesia must be in excess. Boil, filter and dilute to 2000 cc. Add 10 cc. of this magnesium nitrate solution to 5-gm. portions of soil contained in 75-cc. porcelain dishes. Mix soil and magnesium nitrate solution well; evaporate on electric hot plate. Ignite to red heat in electric muffle furnace; add enough water to moisten; loosen and pulverize with pestle without removing from porcelain dish; add 10 cc. of concentrated hydrochloric acid and evaporate to dryness on hot plate. Moisten with water; add 10 cc. of concentrated hydrochloric acid; heat to boiling; and filter and wash on a Hirsch-Buchner funnel, using medium suction, into 250 cc. beakers. Heat to the boiling point on the electric hot plate; add, drop by drop, 5 cc. of a 10-per cent solution of $BaCl_2$; digest 1–2 hours; allow to stand over night or an equivalent length of time; filter on tarred Gooch crucible; ignite and weigh as usual. It is obvious that blank determinations must be made on all reagents used.

One advantage of the magnesium nitrate method is that the phosphorus if wanted, can be determined on the filtrate from the sulfur determination. The method is to neutralize with ammonium hydroxide; acidify with nitric acid; evaporate to about 25 cc.; add 5 to 10 gm. ammonium nitrate; raise the temperature to the desired degree and precipitate the phosphorus with the molybdate solution.

The average sulfur content indicated by 96 determinations made by each of these methods was 0.035 per cent for the magnesium nitrate method and 0.0345 per cent for the sodium peroxide method. This shows that the magnesium nitrate method was as reliable in the aggregate as the sodium peroxide method. It was noticed that the determinations with the magnesium nitrate method gave better duplicates in the hands of different analysts and this made it appear that individual results were therefore more reliable. For this reason the results obtained by the magnesium nitrate method are used in the tables of this report.

The results of the analyses of the soils are found in table 2. Since these samples were taken from all parts of the state, differing greatly in climatic

TABLE 2

Analyses of soils

SAMPLE NO.	COUNTY	CROP	SULFUR	NITROGEN	CARBON	PHOSPHORUS
Humid section—Alfalfa						
			per cent	*per cent*	*per cent*	*per cent*
1768	Brown	Alfalfa 28 years	0.044	0.211	2.28	0.063
1897	Butler	Alfalfa 12 years	0.026	0.161	1.71	0.038
1900	Chase	Alfalfa 25 years	0.032	0.201	2.23	0.039
1874	Dickinson	Alfalfa 20 years	0.028	0.168	1.82	0.048
1877	Dickinson	Alfalfa 20 years	0.026	0.177	2.00	0.044
1880	Dickinson	Alfalfa 25 years	0.032	0.201	2.51	0.091
1882	Dickinson	Alfalfa 15 years	0.021	0.157	2.27	0.052
1890	Harvey	Alfalfa 11 years	0.023	0.161	1.91	0.041
1893	Harvey	Alfalfa 14 years	0.028	0.200	2.31	0.043
1316	Leavenworth	Alfalfa 8 years	0.033	0.163	1.61	0.043
1365	Nemaha	Alfalfa 20 years	0.030	0.170	1.69	0.039
Average			0.029	0.179	2.03	0.049
Humid section—Virgin sod						
1770	Brown	Pasture, blue grass	0.041	0.228	2.86	0.061
1898	Butler	Pasture, native	0.030	0.196	2.35	0.035
1903	Chase	Meadow, blue stem	0.034	0.201	2.48	0.058
1876	Dickinson	Meadow, blue stem	0.026	0.204	2.46	0.048
1879	Dickinson	Native, blue stem	0.038	0.204	2.63	0.061
1881	Dickinson	Native, blue stem	0.035	0.209	3.03	0.087
1891	Harvey	Native, blue stem	0.031	0.189	2.28	0.056
1894	Harvey	Native, pasture	0.031	0.222	2.66	0.045
1319	Leavenworth	Native, blue stem	0.047	0.296	3.76	0.061
1367	Nemaha	Native, pasture	0.036	0.181	1.97	0.038
Average			0.035	0.193	2.65	0.055
Humid section—Cropped to grain						
1769	Brown	Grain, 50 years	0.027	0.160	1.94	0.048
1896	Butler	Corn and wheat, 32 years	0.024	0.139	1.54	0.027
1901	Chase	Corn and wheat, 40 years	0.027	0.133	1.80	0.042
1875	Dickinson	Small grain, 33 years	0.030	0.140	1.82	0.057
1878	Dickinson	Wheat and oats, 40 years	0.023	0.163	2.00	0.048
1883	Dickinson	Wheat and kaffir, 35 years	0.028	0.155	2.06	0.045
1892	Harvey	Wheat and corn, 25 years	0.023	0.140	1.12	0.053
1895	Harvey	Wheat and corn, 43 years	0.022	0.124	1.23	0.036
1317	Leavenworth	Grain, corn 40 years	0.035	0.174	1.87	0.048
1766	Nemaha	Grain, 48 years	0.024	0.102	1.13	0.040
Average			0.026	0.143	1.65	0.044

TABLE 2—*Continued*

SAMPLE NO.	COUNTY	CROP	SULFUR	NITROGEN	CARBON	PHOSPHORUS
Subhumid section—Alfalfa						
			per cent	*per cent*	*per cent*	*per cent*
1884	Barton	Alfalfa, 15 years	0.031	0.159	1.91	0.054
1771	Mitchell	Alfalfa, 21 years	0.044	0.204	2.42	0.075
1774	Mitchell	Alfalfa, 21 years	0.076	0.269	3.23	0.069
1779	Mitchell	Alfalfa, 23 years	0.037	0.160	1.26	0.079
1783	Osborne	Alfalfa, 20 years	0.041	0.184	2.30	0.060
1787	Osborne	Alfalfa, 33 years	0.049	0.196	2.22	0.070
1887	Pawnee	Alfalfa, 28 years	0.035	0.221	2.56	0.053
Average			0.045	0.199	2.27	0.066
Sub-humid section—Virgin sod						
1885	Barton	Native grass	0.021	0.106	1.28	0.052
1773	Mitchell	Native pasture	0.042	0.238	3.14	0.063
1776	Mitchell	Native, grass	0.085	0.290	2.89	0.105
1778	Mitchell	Native grass	0.034	0.180	1.87	0.068
1780	Mitchell	Native, pasture	0.040	0.153	1.78	0.055
1784	Osborne	Native, pasture	0.051	0.250	3.22	0.068
1786	Osborne	Native, grass	0.053	0.232	3.71	0.106
1789	Osborne	Native, timber	0.039	0.220	3.11	0.100
1888	Pawnee	Buffalo grass	0.033	0.214	2.53	0.059
Average			0.044	0.209	2.61	0.075
Sub-humid section—Cropped to grain						
1886	Barton	Wheat and corn, 30 years	0.022	0.137	1.68	0.047
1772	Mitchell	Wheat and corn, 30 years	0.040	0.180	2.18	0.072
1775	Mitchell	Wheat and corn, 23 years	0.091	0.186	2.09	0.096
1777	Mitchell	Wheat and corn, 30 years	0.033	0.129	1.42	0.065
1781	Mitchell	Wheat and corn, 40 years	0.026	0.115	1.34	0.057
1782	Osborne	Wheat and corn, 40 years	0.037	0.134	1.66	0.067
1785	Osborne	Wheat and corn, 35 years	0.048	0.211	2.65	0.103
1788	Osborne	Corn, 40 years	0.049	0.143	1.77	0.080
1789	Pawnee	Corn and kafir, 20 years	0.036	0.201	2.11	0.093
Average			0.042	0.160	1.88	0.076
Semi-arid section—Alfalfa						
1299	Finney	Alfalfa, 20 years	0.034	0.168	1.61	0.076
1303	Finney	Alfalfa, 27 years	0.059	0.200	1.93	0.085
1304	Finney	Alfalfa, 27 years	0.034	0.178	1.72	0.064
1306	Finney	Alfalfa, 30 years	0.055	0.192	2.04	0.078
1310	Ford	Alfalfa, 30 years	0.036	0.210	2.08	0.082

TABLE 2—*Concluded*

SAMPLE NO.	COUNTY	CROP	SULFUR	NITROGEN	CARBON	PHOSPHORUS
		Semi-arid section—Alfalfa—Continued				
			per cent	*per cent*	*per cent*	*per cent*
1313	Ford	Alfalfa, 10 years	0.027	0.156	3.66	0.082
1794	Trego	Alfalfa, 20 years	0.025	0.131	1.06	0.072
1798	Gove	Alfalfa, 15 years	0.036	0.210	2.23	0.100
1800	Gove	Alfalfa, 30 years	0.034	0.184	1.90	0.093
1802	Gove	Alfalfa, 20 years	0.030	0.133	1.44	0.098
1806	Sheridan	Alfalfa, 20 years	0.033	0.187	1.83	0.093
1809	Sheridan	Alfalfa, 20 years	0.027	0.153	1.63	0.084
1811	Wallace	Alfalfa, 25 years	0.046	0.182	1.76	0.099
Average			0.037	0.176	1.91	0.085
		Semi-arid section—Virgin sod				
1300	Finney	Buffalo grass	0.027	0.137	1.94	0.077
1302	Finney	Buffalo grass	0.031	0.135	1.68	0.082
1308	Finney	Buffalo grass	0.015	0.099	0.89	0.068
1315	Ford	Buffalo grass	0.032	0.152	1.74	
1797	Gove	Buffalo grass	0.027	0.154	1.84	0.084
1799	Gove	Buffalo grass	0.032	0.188	2.16	0.086
1803	Gove	Buffalo grass	0.029	0.116	1.27	0.080
1807	Sheridan	Buffalo grass	0.034	0.182	2.27	0.064
1810	Sheridan	Buffalo grass	0.028	0.157	1.82	0.098
1812	Wallace	Buffalo grass	0.033	0.151	1.89	0.080
Average			0.028	0.149	1.78	0.080
		Semi-arid section—Cropped to grain				
1301	Finney	Wheat and Sudan grass, 20 years	0.042	0.134	1.76	0.070
1305	Finney	Wheat and grain, 27 years	0.019	0.079	1.04	0.053
1307	Finney	Wheat and grain, 27 years	0.016	0.097	0.98	0.062
1312	Ford	Wheat and grain, 30 years	0.031	0.136	1.82	0.075
1314	Ford	Wheat and grain, 30 years	0.021	0.118	1.48	0.063
1795	Trego	Wheat, unknown	0.019	0.160	1.84	0.073
1796	Gove	Wheat, 30 years	0.019	0.128	1.35	0.105
1801	Gove	Wheat and grain, 15 years	0.025	0.121	1.21	0.090
1804	Gove	Wheat, 30 years	0.033	0.118	1.31	0.099
1808	Sheridan	Wheat and grain, 20 years	0.024	0.118	1.14	0.082
Average			0.025	0.121	1.39	0.077

conditions, it was thought best to group the results under three heads. Under the humid section are classed all soils taken where the average rainfall is 30 inches or more; under the sub-humid section, those taken where the average rainfall is between 30 and 22 inches; and under the semi-arid section, those taken where the average rainfall is less than 22 inches. The percentages of nitrogen, carbon and phosphorus are taken from the study last mentioned (9).

The results on sulfur showed great variation in different groups but not more so than the results on nitrogen or phosphorus. It is only when the average for each group within the different sections are considered that any conclusions can be drawn. These averages were used to calculate the pounds per acre assuming two million pounds for the surface seven inches. The results obtained by this calculation are found in table 3.

In examining the results of the sulfur determinations it was noticed that three soils from the semi-arid section had an abnormally high content of sulfur. Soil 1309 was 0.149 per cent sulfur. This sample was taken on bottom soil near the Arkansas river which had been flooded occasionally. Soil 1311 was 0.065 per cent sulfur. This sample was taken on virgin sod near the railroad. Soil 1805 was 0.111 per cent sulfur. This sample was taken on bottom soil in virgin sod that received seepage water from surrounding higher land. These facts are mentioned to show that occasionally soils are found that have an abnormal amount of sulfur. The results from these three samples were not included in table 2.

TABLE 3

Average plant food elements per acre in the surface soil

SECTION	CROP	SULFUR	NITROGEN	CARBON	PHOSPHORUS
		pounds	*pounds*	*pounds*	*pounds*
Humid	Alfalfa	580	3,580	40,600	980
	Virgin sod	700	3,860	53,000	1,100
	Grain	520	2,860	33,000	880
Sub-humid	Alfalfa	900	3,980	45,400	1,320
	Virgin sod	880	4,180	52,200	1,500
	Grain	840	3,200	37,600	1,520
Semi-arid	Alfalfa	740	3,520	38,200	1,700
	Virgin sod	560	2,980	35,600	1,600
	Grain	500	2,420	27,800	1,540

In the humid sections, soils in alfalfa had 120 pounds less sulfur per acre than soils in virgin sod, while the soils cropped to grain had 180 pounds less. There were, apparently, losses of 17 and 25 per cent respectively, of the original sulfur-content.

In both the sub-humid and semi-arid sections, the soils cropped to alfalfa had more sulfur than the soils in virgin sod. The greatest difference was in the semi-arid section. This must mean that the deep-feeding alfalfa roots transferred large amounts of sulfur from the subsoil to the surface soil. Most of the organic matter of alfalfa roots is found in the surface foot of the soil. A large amount of alfalfa leaves are annually dropped on the soil where they decay. Accumulation of organic matter, rich in protein material would increase the sulfur content of the surface soil. The average sulfur content of the soil cropped to grain was less than the sulfur content of the soils in virgin soil, but the loss of sulfur due to grain-cropping was small in the sub-humid and semi-arid sections.

In the humid section, the soils cropped to grain had 1000 pounds less nitrogen per acre than the soils in virgin sod, or a loss of about 26 per cent. The loss of nitrogen parallelled the loss of sulfur. The soils in alfalfa had less nitrogen than the soils in virgin sod, but the difference was only 8 per cent.

In the sub-humid section, the nitrogen content of the soils in alfalfa and the soils in virgin sod differed by only 200 pounds per acre, or only 5 per cent. Alfalfa, therefore, practically maintained the nitrogen content of the soil.

TABLE 4

Relative sulfur-content of crops and phosphorus based on averages of several published analyses

CROP	AVERAGE CROP YIELD	SULFUR IN CROP		PHOSPHORUS IN CROPS	
		per cent	*pounds*	*per cent*	*pounds*
Alfalfa hay	5,000 lbs.	0.287	14.25	0.24	12.00
Corn, grain	20 bu.	0.170	1.90	0.30	3.36
Corn, stover	200 lbs.	0.126	1.51	0.20	2.40
Total corn crop			3.41		5.76
Oats, grain	25 bu.	0.189	1.51	0.35	2.80
Oats, straw	1,260 lbs.	0.195	2.44	0.09	1.13
Total oats crop			3.95		3.93
Wheat, grain	15 bu.	0.170	1.53	0.37	3.33
Wheat, straw	1,250 lbs.	0.119	1.79	0.06	0.75
Total wheat crop			3.32		4.08

The soils in virgin sod has 980 pounds more nitrogen than the soils cropped to grain, or a loss of 23 per cent. This loss was much greater than the loss of sulfur for this section.

In the semi-arid section, the soils in alfalfa had more nitrogen than the soils in virgin sod, which shows that the alfalfa has taken enough nitrogen from the air not only to compensate for that removed in the hay, but to increase the nitrogen-content of the soil. These soils also increased in sulfur due, apparently to the transference of sulfur from the subsoil and the addition in rain. The soils cropped to grain had 560 pounds less nitrogen than the soils in virgin sod, a loss of 19 per cent while the sulfur loss was 11 per cent.

In the central and western part of the state apparently, the additions and losses of nitrogen compensated each other. In the eastern part of the state the losses were greater than the gains.

The changes in carbon-content almost parallelled the changes in nitrogen.

Changes in the organic-content of the soil naturally cause changes in the sulfur-content as well as in the nitrogen-content.

The soils in the humid and sub-humid sections contain a little over one and one-half times as much phosphorus as sulfur. In the semi-arid section, the phosphorus-content is nearly three times that of sulfur.

Table 4 presents the relative content of common farm crops of phosphorus and sulfur. Corn and wheat take more phosphorus from the soil than sulfur while oats take an equal amount of each element. Alfalfa requires more sulfur than phosphorus. Alfalfa removes more sulfur than any other crop. Table 3 shows that less sulfur has been lost from alfalfa fields than from those cropped to grain. This is explained by the transference of sulfur from the subsoil by the deep roots of alfalfa. Also the loss from leaching and washing is less in the field devoted to alfalfa.

Assuming that the average amount of sulfur removed annually by such crops as corn, wheat and oats is 3.5 pounds, the amount removed in 40 years would be 140 pounds. Table 3 shows that more than this amount has been lost from cultivated soils in eastern Kansas. This simply means that the sulfur added in rainfall is not sufficient to compensate for the amount of sulfur lost by leaching.

CONCLUSION

Whether sulfur is now a limiting element in crop production on Kansas soils will have to be determined by fertilizer tests in which sulfur is one of the leading elements. This study has shown that sulfur is decreasing in eastern Kansas soils and that this decrease is proportionate to the amount removed by crops. The amount added by rain is apparently not even sufficient to make good that lost by leaching. The loss of sulfur is greater than phosphorus and somewhat proportionate to the loss of nitrogen. These elements are known to be limiting elements in crop production. If sulfur continues to be lost from the soil in greater amounts than it is supplied it is reasonable to conclude that it also will be a limiting factor in crop production if it is not one already.

SUMMARY

1. In this paper are presented the amounts of sulfur found in 96 Kansas soils, some cropped to grain, some to alfalfa, and some in virgin sod.

2. The use of magnesium nitrate instead of sodium peroxide for determining sulfur in soil is described.

3. In the eastern or humid section of the state, the loss of sulfur in soils cropped to grain is proportionate to the loss in nitrogen. In the central and western parts of the state the loss is small, if any.

4. The amount of sulfur removed from the soil is apparently not compensated for by the amount added in rain. If sulfur continues to be removed faster than it is supplied, the possibility of sulfur being, or becoming, a limiting factor in crop production is indicated by this study.

REFERENCES

(1) Ames, J. W., and Boltz, G. E. 1916 Sulphur in relation to soil and crop. Ohio Agr. Exp. Sta. Bul. 292.

(2) Hart, E. B., and Peterson, W. H. 1911 Sulfur requirements of farm crops in relation to soil and air supply. Wis. Agr. Exp. Res. Bul. 14.

(3) Hilgard, E. W. 1907 Soils, p. 377. MacMillan, New York.

(4) Lipman, J. G., and McLean, H. C. 1916 Sulfur oxidation in soils and its effect on the availability of mineral phosphates. *In* Soil Sci., v. 2, p. 499–538.

(5) McLean, H. C. 1918 The oxidation of sulfur by microörganisms in relation to the availability of phosphorus. *In* Soil Science, v. 5, p. 251–290.

(6) Reimer, F. C., and Tartar, H. V. 1919 Sulfur as a fertilizer for alfalfa in southern Oregon. Ore. Agr. Exp. Sta. Bul. 163.

(7) Shedd, O. M. 1917 Effect of sulfur on different crops and soils. *In* Jour. Agr. Res., v. 11, p. 91–103.

(8) Swanson, C. O., and Miller, R. W. 1917 The sulfur content of some typical Kansas soils and the loss of sulfur due to cultivation. *In* Soil Sci., v. 3, p. 139–148.

(9) Swanson, C. O., and Latshaw, W. L. 1919 Effect of alfalfa on the fertility elements of the soil in comparison with grain crops. *In* Soil Sci., v. 8, p. 1–39.

(10) Wolff, Emil 1871 Aschen Analysen. Wiegand and Hempel, Berlin.

RELATION BETWEEN HEAT OF WETTING, MOISTURE EQUIVALENT AND UNFREE WATER

GEORGE JOHN BOUYOUCOS

Michigan Agricultural Experiment Station

Received for publication July 1, 1922

INTRODUCTION

Water is one of the best indices of the physical characteristics of soil. Texture, structure, colloidal and organic contents, activation of surface, etc., tend to be revealed by the behavior of the soil toward water. Heat of wetting, moisture retentiveness, unfree water, etc., are mainly the resultant and expression of these characteristics.

The question of the relationship of these various soil-water manifestations to each other is obviously of much interest. If the relationship is of positive nature, then it is also of considerable practical importance, because in that case the simplest, most convenient and rapid method could be selected to obtain the other relationships and to make a comparative study of soils.

With these objects in view a comparative study was made of the relationship that exists between a number of soil-water manifestations, but principally those of heat of wetting, moisture-equivalent and unfree water.

METHODS AND PROCEDURE

The heat of wetting was determined according to the method described in a former publication (2).

It consisted of placing about 50 gm. of air-dry soil in a wide glass tube and allowing it to dry in an electrically heated oven at a temperature of 105°C. for about 24 hours. The tube was then taken out, closed with a rubber stopper and allowed to attain the room temperature. After the exact weight and temperature were ascertained, the soil was quickly and carefully poured into a calorimeter containing 100 gm. of water and the heat of wetting was ascertained. Extreme care was taken before mixing to have both the soil and water at exactly the same temperature and very nearly that of the room temperature. In order to be able to convert, if necessary, the rise of temperature into heat calories, the water-equivalent of the calorimeter was determined. This was found to be 25 gm. of water. The specific heat of soils was about .200 for the mineral soils and about .300 for the organic soils.

The unfree water was measured by means of the dilatometer method already described (1) with the modification of washing the soils free of soluble salts before their unfree water was measured. This was done in order to eliminate any effect of soluble salts on the unfree water. Two different moisture contents were employed for each soil, one was at about the optimum stage and the other at the proportion of 20 gm. of soil to 15 cc. of water. Before the final measurement was made, the soils were subjected to freezing and thawing once or twice. The soils were then placed at a temperature of −1.5°C. and allowed to come to equilibrium and the volume on the dilatometer stem recorded. They were then immersed at a temperature of about −12°C. for about fifteen minutes, and then returned to the original temperature.

After equilibrium was attained, the volume on the dilatometer stem was again read and from the two readings the unfree water could be calculated. The term unfree water is used in this paper to designate the soil water remaining unfrozen at the temperature of slightly below zero. All the water which freezes very readily at or very near zero is designated as free water. The free water is beyond the direct influences of the soil and freezes like water in mass, while the unfree water is under the direct influences of the soil and consequently is not at liberty to freeze as the free water.

For the moisture-equivalent the Briggs-McLane apparatus was used. The machine was regulated to run at a speed of about 2450 revolutions per minute and was allowed to run for 40 minutes. Thirty grams of the finer textured types of soil were used, 30 gm. of sand, and 10 to 15 gm. of the peats and muck.

RESULTS

The experimental results obtained on the heat of wetting, unfree water and moisture equivalent on a number of different types of soils, and the relationship that exists between these different soil-water manifestations, are shown in the adjoining table.

There is a rather close relationship in the various soils between the unfree water and the heat of wetting. The ratio ranges only from 0.0408 in soil 20 to 0.0675 in soil 1 with an average ratio of 0.05305 for all of the soils.

It would appear that there is a tendency for soils with much organic matter to give a smaller ratio than soils with very little or no organic matter. But the organic matter content, cannot be entirely responsible for this difference because peat and muck which represent the extreme organic soils have a ratio as large as the mineral soils with no or very little organic matter. A more satisfactory explanation for the difference seems to be found in the hypothesis that among soils causing about the same amount of water to become unfree, some possess a more activated surface, which results in more heat of wetting. Experimental results along this line will be presented in greater detail in a subsequent paper.

Considering next the ratio of moisture-equivalent and heat of wetting it is seen that there is not a very close relationship between these two factors in the various soils. The ratio ranges from 0.0953 in soil 20 to 0.3233 in soil 5 with an average ratio of 0.1685 for all of the soils. The ratio is quite close in many of the soils, but in others it varies considerably.

It is not surprising that there is no closer relationship between the heat of wetting and moisture-equivalent, because besides the naturally different specific properties of the various soils which would affect these two factors differently, the moisture-equivalent method does not give a true and an exact equivalent of moisture for all soils. In very fine textured and colloidal soils which allow the water to get out only with much difficulty and especially when compacted by centrifuging, the moisture-equivalent is considerably greater than it should be in comparison with that in coarse textured and porous soils. In some of the clay soils, for instance, the moisture-equivalent is as much as 25 per cent above what it should be. Results obtained by the freezing point method indicate that with some soils the moisture-equivalent obtained is true but with others it is too high. When the moisture-equivalent, therefore, is not absolutely true in all of the different soils, its relationship with the heat of wetting would tend to vary accordingly. But even if the moisture-equivalent, were absolutely true in all the soils, probably its relationship with the heat of wetting would not be exceedingly close in the various soils, because the two factors are affected differently by soil properties and conditions.

TABLE 1

Relationship between heat of wetting, unfree water and moisture-equivalent

SOIL NUMBER	SOIL TYPE	HEAT OF WETTING	UNFREE WATER	MOISTURE EQUIVALENT	$\frac{U.W.}{H.W.}$*	$\frac{M.E.}{H.W.}$†	$\frac{M.E.}{U.W.}$‡
		°C.	per cent	per cent			
1	Sand	0.2	1.35	3.8	0.0675	0.1900	2.815
2	California Handford sandy loam	0.8	5.2	15.0	0.0650	0.1875	2.885
3	Ohio silt loam	0.7	4.6	21.8	0.0657	0.3114	4.740
4	Ohio silt loam	0.7	3.6	21.3	0.0437	0.3043	5.917
5	Cornell silt loam	0.9	5.25	29.1	0.0583	0.3233	5.541
6	Pennsylvania silt loam	0.9	5.0	28.9	0.0555	0.3211	5.780
7	Pennsylvania silt loam	1.1	7.0	29.2	0.0636	0.2655	5.600
8	Rhode Island sandy loam	1.1	6.0	21.7	0.0545	0.1972	3.617
9	Michigan silt loam	1.65	10.20	25.5	0.0618	0.1546	2.500
10	Wisconsin Superior clay	1.95	10.75	28.2	0.0548	0.1446	2.635
11	California Capay clay	2.0	11.1	33.5	0.0555	0.1675	3.020
12	Michigan clay loam	2.2	13.6	30.2	0.0618	0.1373	2.221
13	California Willows clay	2.2	12.9	32.2	0.0586	0.1464	2.496
14	Minnesota Clyde silt loam	2.55	11.0	29.8	0.0431	0.1170	2.710
15	Minnesota black surf	2.85	14.0	36.5	0.0491	0.1281	2.607
16	Illinois clay loam	2.9	15.2	36.3	0.0524	0.1252	2.338
17	Minnesota Fargo clay	3.0	16.0	39.0	0.0533	0.1300	2.438
18	Iowa Carrington clay loam	3.4	16.0	36.5	0.0472	0.1074	2.282
19	Illinois clay loam	3.5	14.3	35.8	0.0408	0.1023	2.504
20	Illinois black clay loam	3.5	14.7	34.0	0.0420	0.0971	2.313
21	California Merced clay	4.0	17.8	40.2	0.0445	0.1005	2.258
22	California Stockton clay adobe	4.1	18.7	49.5	0.0456	0.1207	2.647
23	Wisconsin black clay loam	4.9	20.9	46.7	0.0426	0.0953	2.235
24	Muck	8.0	37.0	95.5	0.0462	0.1194	2.581
25	Muck	12.6	67.0	147.0	0.0532	0.1167	2.194

* Percentage of unfree water divided by rise of temperature of 50 gm. of soil in 100 gm. of water.

† Moisture-equivalent divided by rise of temperature of 50 gm. of soil in 100 gm. of water.

‡ Moisture-equivalent divided by percentage of unfree water.

It was originally thought that there might be a rather close relationship between the heat of wetting and moisture-equivalent since both factors were considered to be controlled principally by three properties, namely, texture, colloidal and organic matter content. It is evident from the experimental results obtained, however, that there is at least one more property coming into play which affects the results appreciably, namely the activation or nature of the surface.

The ratio between the unfree water and the moisture-equivalent shows that there is not a very close and consistent relationship between the two factors. The ratio ranges from 2.194 in soil 25 to 5.917 in soil 4 with an average ratio of 3.1456.

Comparison of the three ratios with one another shows, also, that there is no very close and consistent parallelism or relation between any two ratios.

SUMMARY

In this paper are presented the results of an investigation conducted to ascertain the relationships that exist between heat of wetting, unfree water, and moisture-equivalent of soils. The experimental results show that there is a close and consistent relationship between the heat of wetting and the unfree water, but there appears to be no close and regular relationship between the heat of wetting and moisture-equivalent or between unfree water and moisture-equivalent.

Evidences obtained go to indicate that the moisture-equivalent method does not give a true and absolutely equivalent moisture in all of the various soils. Some of the fine textured and colloidal soils contain considerably more moisture than their true moisture-equivalent.

REFERENCES

(1) Bouyoucos, George J. 1917 Classification and measurement of different forms of water in the soil by means of the dilatometer method. Mich. Agr. Exp. Sta. Tech. Bul. 36.

(2) Bouyoucos, George J. 1918 Relationship between the unfree water and the heat of wetting of soils and its significance. Mich. Agr. Exp. Sta. Tech. Bul. 42.

SULFUR AS A FERTILIZER FOR COTTON

E. B. REYNOLDS AND A. H. LEIDIGH

Texas Agricultural Experiment Station

Received for publication July 6, 1922

In 1920 the Texas Agricultural Experiment Station conducted at Substation No. 5, Temple, Texas, preliminary experiments[1] with sulfur to determine its value as a fertilizer for cotton and also to ascertain whether it would be possible to control or prevent the Texas root-rot of cotton, *Phymatotrichum omnivorum* (Shear) Duggar by the use of sulfur. Acid phosphate alone and in combination with sulfur was included in part of this work, although, in previous experiments with fertilizers, phosphatic materials had never given large increases in yield.

This preliminary work was conducted on Abilene clay soil, which is generally considered as not needing lime or phosphorus. This soil has been cropped continuously to cotton for the last ten years.

The plots were one-twentieth of an acre in size, with rows 36 inches apart. The fertilizer materials were applied in the drill on March 27, and the cotton was planted on April 10.

Table 1 gives the plan of the experiment, and the yield of lint and seed cotton to the acre.

It will be noted that sulfur applied at the rate of 1000 pounds to the acre made a much larger yield than any other treatment. Sulfur in combination with acid phosphate produced larger yields than acid phosphate alone. Sulfur, however, at the rate of 500 pounds to the acre, on plot 4, produced a smaller yield than did any other treatment in the test. The average yield of the three plots which received sulfur was 374.74 pounds of lint cotton to the acre; of the plots which received sulfur and phosphorus, 359.08 pounds; of the plot which received phosphorus alone, 259.76 pounds; and the average yield of the soil checks was 276.63 pounds to the acre. In general, sulfur increased the yield, while acid phosphate did not.

The cotton on all plots made comparatively small vegetative growth, but the plants on plot 2 were, as an average, about 3 or 4 inches taller than the plants on any other plot in the experiment. The average height of the plants on the plots which were treated with sulfur was 2–3 inches greater than the height of the plants on the plots which did not receive sulfur. The foliage on plot 2 was darker green than the foliage on the other plots.

Texas root-rot[2] was present uniformly on all plots, and by actual count affected 90–95 per cent of the plants. The plants on plot 2, however, were not affected so early as the plants on the other plots. This fact probably explains the great increase in yield of plot 2 over the yields of the other plots.

In 1920, in another experiment with sulfur as a fertilizer for cotton on a shallow phase of Houston clay soil on the farm of S. H. Cater near Substation No. 5, sulfur was applied alone at the rates of 100, 300, and 600 pounds to the acre in the drill, on March 25, and the cotton was planted on April 8. The size of the plots was one-eighth of an acre. The results of this experiment are given in table 2.

---

[1] Credit is due Mr. D. T. Killough, Superintendent, for conducting the field work and collecting the yield data reported in this paper.

[2] The root-rot investigations are being conducted by Dr. J. J. Taubenhaus, Chief, Division of Plant Pathology and Physiology. The authors are indebted to him for the notes on root-rot reported in this paper.

Table 2 shows that the yields of cotton increased with the amount of sulfur applied, although the increase in yield was not directly proportional to the increase in the amount of sulfur used.

Apparently, sulfur had no effect on the root-rot for the disease was uniformly distributed on all the plots, irrespective of the treatments. Very little difference in growth of the cotton could be noted on the various plots.

TABLE 1

Yield per acre of lint and seed cotton on plots in experiment with sulfur and acid phosphate, 1920

PLOT NUMBER	TREATMENT		YIELD PER ACRE	
	Material	Amount	Lint	Seed cotton
		lbs.	*lbs.*	*lbs.*
1	Nothing-check		275.04	720.00
2	Sulfur	1000	527.35	1380.50
3	Sulfur	500		
	Acid phosphate	400	368.63	965.00
4	Sulfur	500	234.93	615.00
5	Nothing-check		295.09	772.50
6	Sulfur	50		
	Acid phosphate	400	349.53	915.00
7	Acid phosphate	400	259.76	680.00
8	Sulfur	50	361.94	947.50
9	Nothing-check		259.76	680.00
Average of checks			276.63	724.16

TABLE 2

Yield per acre of lint and seed cotton in the sulfur fertilizer experiment, 1920

PLOT NUMBER	TREATMENT	YIELD PER ACRE	
		Seed cotton	Lint
		lbs.	*lbs.*
1	Nothing-check	496	187.43
2	100 pounds sulfur	608	229.76
3	300 pounds sulfur	632	238.83
4	600 pounds sulfur	776	293.25

The results in 1920, presented in tables 1 and 2, suggested that it would be advisable to establish a more comprehensive experiment with sulfur with the idea of securing additional information on its use as a fertilizer and its effect on the soil, such as the lime requirement, hydrogen-ion concentration, and bacterial activities.

Accordingly, an experiment was outlined, which comprised applications of sulfur and lime, alone and in combination, in amounts varying from 500 pounds to 10,000 pounds to the acre. This work is conducted on Simmons clay and Abilene clay at Substation No. 5, Temple, Texas. These soils are classified as high terrace soils by the Bureau of Soils, and are, as a rule, well supplied with lime and phosphorus. The hydrogen-ion concentration of the Abilene clay soil was pH 8.00 at the beginning of the experiment.

Two series of 16 plots each are included in the experiment. Series 1 comprises a three-year rotation of corn, small grain (wheat or oats) followed by cowpeas as a catch crop, and cotton. Series 2 receives the same treatment as series 1, but it includes continuous cotton instead of cotton in rotation. Series 2 is conducted on soil which has grown cotton for the last eleven years. In series 1, the treatments are applied to the cotton only; thus each of the three acres in the rotation will receive sulfur only once in three years, while in series 2 the land receives the treatments every year. The size of plots in the rotation, series 1, is one-twenty-second of an acre and in series 2, one-forty-fourth acre.

Lime and the sulfur were applied broadcast on April 20, 1921 and the cotton was planted on May 4. Inoculated sulfur and pure hydrated lime were used in this experiment.

TABLE 3

Germination of cotton planted in soil treated with sulfur, and hydrogen-ion concentration of the soil on April 1, 1921

BOX NUMBER	SULFUR APPLIED PER ACRE	GERMINATION			REACTION APRIL 1
		First	Second	Third	
		per cent			*pH*
1	Nothing-check	76	Normal	Normal	7.00
2	500 pounds	84	A few	None	
3	1000 pounds	82	A few	None	
4	2500 pounds	66	None	None	2.20
5	5000 pounds	70	None	None	2.20
6	10000 pounds	76	None	None	2.20

In outlining this experiment it was thought that the heavy applications of sulfur would retard and perhaps prevent the normal development of the cotton plant in the field, which condition, in this case, was desired.

In order to obtain some information on this point some tentative tests were made in the laboratory. In these tests sulfur was applied at the rates of 500, 1000, 2500, 5000, and 10000 pounds to the acre on sandy loam soil, in wooden germinating boxes, on February 19. The boxes were 18 inches long, 8 inches wide, and 4 inches deep. The results of this test are presented in table 3.

Germination was normal in all boxes in the first planting. Cotton seeds were planted again in the same boxes on March 14, 1921. The seeds in box 1, check, germinated normally by March 19 and a few seeds germinated in boxes 2 and 3. There was no germination in the other boxes. The germination test was repeated again on March 23 and the seeds on the check germinated in a normal manner while no germination occurred in the other boxes. These results are in accord with those of Shedd (3).

The hydrogen-ion concentration of the soil with the three heaviest applications in the boxes was determined tentatively on April 1, 1921 by the method suggested by Wherry (4). The results of this determination are shown in the last column of table 3. It should be kept in mind that the soil used in this laboratory test was entirely different from the soil on which the field experiment was conducted.

Unfortunately the laboratory work planned in connection with the field work could not be carried out, and the yield data are presented for what they may be worth.

The amounts of lime and sulfur applied to the acre and the yields of both series of plots are given in table 4.

It will be noted from table 4 that the highest yield of both lint and seed cotton in the rotation, series 1, resulted from the application of 5000 pounds of lime. The lowest yield in series 1 was obtained from the plot which received the largest amount of sulfur, 10,000 pounds. In every case, with one exception, the plots which received sulfur alone produced smaller yields than did the corresponding plots which received both lime and sulfur in equal quantities.

TABLE 4

Yields per acre of lint and seed cotton in experiment with lime and sulfur, 1921

PLOT NUMBER	TREATMENT	ROOT-ROT		SEED COTTON		LINT	
		Series 1 (Rotated)	Series 2 (Not rotated)	Series 1 (Rotated)	Series 2 (Not rotated)	Series 1 (Rotated)	Series 2 (Not rotated)
		per cent	*per cent*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
1	Nothing-check	10	8	858.00	792.00	290.54	271.10
2	500 pounds lime	6	10	864.00	660.00	291.60	225.09
3	500 pounds sulfur	2	12	800.00	550.00	271.47	187.37
4	500 pounds lime and 500 pounds sulfur	4	25	880.00	418.00	299.59	143.02
5	2500 pounds lime	3	35	887.50	396.00	300.66	136.62
6	Nothing-check	2	50	880.00	374.00	300.04	129.03
7	2500 pounds sulfur	4	95	704.00	27.50	240.38	9.48
8	2500 pounds lime and 2500 pounds sulfur	3	92	770.00	24.05	262.92	8.29
9	5000 pounds lime	0	86	902.00	108.62	306.68	37.47
10	5000 pounds sulfur	1	78	704.00	110.00	239.79	37.71
11	Nothing-check	5	70.5	748.00	286.00	254.38	97.00
12	5000 pounds lime and 5000 pounds sulfur	4	45	671.00	275.00	229.24	92.73
13	10000 pounds lime	T	18	814.00	396.00	277.38	132.58
14	10000 pounds sulfur	T	8.5	638.00	396.00	217.50	133.53
15	10000 pounds lime and 10000 pounds sulfur	T	5	792.00	484.00	270.03	162.23
16	Nothing-check	5	4	682.00	594.00	232.91	198.52
Average of checks				792.00	511.50	269.46	173.91

This is probably due to the fact that the acidity which was formed by the oxidation of sulfur was injurious to the cotton plant, and consequently reduced the yield. The lime which was applied with the sulfur neutralized some of the acid which developed, and as a result, improved the soil conditions, which resulted in an increase in yield as compared with the corresponding plots which received sulfur alone. Lipman, Prince, and Blair (2) found that applications of 1000, 2000 and 4000 pounds of sulfur made sharp increases in the hydrogen-ion concentration and lime requirement of the soil after the sulfur had been in the soil about six weeks. In our experiment it seems quite probable that similar increases in the hydrogen-ion concentration and lime requirement occurred, and that the lime which was present in the soil neutralized part of the acidity and consequently increased the yields as mentioned above.

Quite different results were secured with the non-rotated cotton, series 2. There is apparently no correlation between the yield of cotton and the quantities of lime and sulfur applied. This result is due, partially at least, to the irregular distribution of root-rot on this series. If the percentages of root-rot on series 1 and series 2 are compared, it seems that the irregular distribution of root-rot overshadowed the influence of the fertilizer treatments on series 2. Previous experiments (1, p. 12–13) with rotations and continuous culture of cotton at Substation No. 5 show that the root-rot of cotton may be controlled to a great extent by the proper use of rotations and cultural methods.

While the tests in the laboratory showed that the heavy applications of sulfur formed enough acid to prevent germination and growth, no apparent injury resulted from the heavy applications in the field. The soil on the plots, as stated previously, is fairly well supplied with lime, having a pH value of 8.00 at the beginning of the experiment. The foliage on the plots which received 5000 and 10000 pounds of sulfur alone was lighter green in color than the foliage on the other plots.

Shedd (3) found that 5000 to 7000 pounds of sulfur to the acre killed cabbage, mustard, and radishes, but the soil he used was slightly acid.

Lipman, Prince, and Blair (2) applied sulfur to the soil at the rates of 200, 500, 1000, 2000 and 4000 pounds to the acre to barley and soybeans (as a residual crop after the barley). The barley germinated fairly well on all the plots, but there was evidence of injury with 1000 pounds of sulfur. Practically all of the plants on the plots that received 4000 pounds had been killed before harvest. One thousand pounds of sulfur depressed the germination of soy beans, and there were very few plants on the plots that received 2000 and 4000 pounds of sulfur.

SUMMARY

In 1920 cotton was grown on plots to which sulfur was applied at the rates of 50, 500, and 1000 pounds to the acre. Acid phosphate was also applied alone and in combination with sulfur at the rates of 400 pounds to the acre.

In general, sulfur increased the yield of cotton. The average yield of the three plots which received sulfur alone was 374.74 pounds lint cotton to the acre; of the plots which received acid phosphate and sulfur, 359.08 pounds; of the plot which received acid phosphate alone, 259.76 pounds; while the average yield of the soil checks was 276.63 pounds to the acre. The cotton on the plot which received 1000 pounds sulfur made a larger vegetative growth and had darker green leaves than the cotton on the other plots.

Another experiment was conducted in 1920 in which sulfur was applied to the soil at the rates of 100, 300, and 600 pounds to the acre. The yields of cotton increased with the increase in the amount of sulfur applied.

The field work in 1921 included applications of sulfur and lime, alone and in combination, in amounts varying from 500 to 10,000 pounds to the acre. In this experiment the largest yield of cotton resulted from the plot which received 5000 pounds of lime. Apparently the sulfur alone had a tendency to depress the yield. Where lime was applied with the sulfur, in the rotated series, the yield of cotton was larger than the yields of the plots which received corresponding amounts of sulfur. Apparently lime mitigated the injurious effect of the heavy applications of sulfur by neutralizing the acid formed by the oxidation of the sulfur.

In these experiments sulfur has neither prevented nor controlled the development of root-rot of cotton.

This work with some modification is being continued.

REFERENCES

(1) Killough, D. T. 1917 Progress report, Substation No. 5, Temple, Texas, 1910–1914. Tex. Agr. Exp. Sta. Bul. 215.

(2) Lipman, J. G., Prince, A. L., and Blair, A. W. 1921 The influence of varying amounts of sulfur in the soil, on crop yields, hydrogen-ion concentration, lime requirement and nitrate formation. *In* Soil Sci., v. 12, no. 3, p. 197–207.

(3) Shedd, O. M. 1914 The relation of sulfur to soil fertility. Ky. Agr. Exp. Sta. Bul. 188.

(4) Wherry, E. T. 1920 Determining soil acidity and alkalinity by indicators in the field. *In* Jour. Wash. Acad. Sci., v. 10, p. 217.

ADSORPTION OF PLANT FOOD BY COLLOIDAL SILICA

R. C. WILEY AND NEIL E. GORDON

University of Maryland

Received for publication July 13, 1922

INTRODUCTION

The question as to the exact manner in which soil colloids retain plant food, whether by chemical reaction or by physical adsorption, is one that has never been settled.

Biederman (1) was one of the first to emphaize the importance of absorption by soils. He felt that the ability of the soil to take up soluble fertilizers and then give them back to the soil was of great significance to practical agriculture as well as extremely interesting from a philosophical point of view. One question he raised was "In what form do plants obtain their nourishment from the soil?"

Salomon (7) determined the adsorption of calcium by various soils and soil constituents and among the latter was hydrated silicic acid. He added enough ammonia to neutralize the nitric acid of the calcium nitrate. Under these conditions he found that the adsorption of the calcium by the hydrated silicic acid increased with the concentration of the calcium nitrate.

Frey (2) pointed out that it is very probable that the absorptive power of soil is proportional to the silicate bases such as alumina, ferric oxide, etc. He worked to a large extent with soils as a whole and hence his problem became very complex.

Van Bemmelen (8) stated that the soil contains colloids such as iron oxide, silicic acid and humus. He believed that these colloids absorbed some of the salts from solution, but how this was accomplished he was uncertain.

Some more recent workers (6) have shown more plainly that there are definite soil colloids and have succeeded in mechanically separating a large part of the colloidal material from soils. The composition of these colloids vary with different soils.

In most of the work thus far done on soil colloids, the systems with which investigators have worked have been so complex that it has not been possible to say whether one had physical absorption or chemical combination. A preliminary paper by Gordon and Starkey (4) contrasts the different soil colloids in their power of absorbing salts and in making them available for plant food. The purpose of the present invesitgation was to further study absorption by silica in order to find what power colloidal silica had of holding the salts commonly used in plant nutrition work. Determinations were made for the adsorption of both negative and positive ions to find out just how much, if any, splitting of the molecule occurred during adsorption.

MATERIAL

Since the authors believed that the soil colloids at times existed as a hydrogel and at other times as a hydrosol (3), it was decided to try the adsorption of the salts by both the hydrogel and hydrosol of silica.

Silica gel was bought of the Davidson Chemical Company, and was washed with distilled water until the wash water gave no test for sulfates. When the washing was finished and the hydrogel drained, it contained 25–35 per cent water. The exact water-content was determined on each portion used. The hydrosol was made in the usual way by treating sodium silicate with dilute hydrochloric acid and dialyzing until free from electrolytes. In this way a very stable hydrosol was obtained.

The salts used in the work were potassium nitrate, potassium sulfate, potassium acid sulfate and the calcium and magnesium salts of the same acids. These salts were recommended by the National Research Council and put out by J. T. Baker and Company.

EXPERIMENTAL WORK

One-gram portions of the hydrogel were weighed into sixty glass stoppered bottles of about 400-cc. capacity. While the hydrogel was being weighed into bottles, a sample of it was taken for moisture determination. Two 100-cc. portions of the solutions of the salts to be tested were added to the bottles containing the gel and the bottles were sealed and shaken until equilibrium was established.

In the analytical work the greatest precautions were taken to insure the most accurate and comparable results. A brief outline of the analytical work is given.

A portion of the solution which was to be analyzed for nitrogen was measured with a pipette into a flask. Five grams of iron, 200 cc. of water and 10 cc. of concentrated sulfuric acid were added to the flask, and the mixture carefully heated for thirty minutes. It was then cooled and the nitrogen distilled over as in the Kjeldahl method.

Sulfur was determined in the usual way: that is, by precipitating as barium sulphate, igniting and weighing.

Phosphorus was determined by the volumetric method as adopted by the Association of Official Agricultural Chemists.

Potassium was determined by the method of the Association of Official Agricultural Chemists.

Calcium was precipitated as calcium oxalate. After washing the precipitate it was dissolved in sulfuric acid and titrated against potassium permanganate in the usual way. One cubic centimeter of the potassium permanganate solution equalled 0.0001 gm. calcium.

Magnesium was determined by precipitating it as magnesium ammonium phosphate, washing the precipitate carefully with dilute ammonium hydroxide solution, drying it on the filter paper at 60°C., dissolving it in a known amount of standard acid and titrating back with standard alkali. One cubic centimeter of the standard acid was equal to 0.0001 gm. magnesium. All determinations were run in duplicate and each result reported is the average of the two.

EXPERIMENTAL RESULTS

Table 1 shows the data obtained in testing the adsorption of the hydrogel from solutions of potassium, calcium and magnesium nitrate and potassium, calcium and magnesium sulfate.

The table shows some results that were anticipated as well as some that were not. One would expect positive adsorption in wet gel. Columns 5 and 10 show such results. In the dry gels there was negative adsorption except in the case of magnesium and the sulfate of potassium. The negative adsorption might indicate one of three things: (*a*) that all the water of hydration did not act as a diluent; (*b*) that the water was more strongly adsorbed than the salt, or (*c*) that we have fine capillaries with which to deal, and, if so, according to Mathieu (5) the solution would be more concentrated due to the solution in the capillaries being less concentrated. The ions of the respective salts are absorbed in practically equivalent quantities with the exception of potassium sulfate.

In table 2 the results are given where acid phosphate solutions of calcium, magnesium and potassium of varying concentrations are used with the hydrogel of silica. The columns are arranged similarly to those in table 1.

In table 2 as in table 1 we find positive adsorption with the wet gel, but with the gel when the water of hydration is figured as water of dilution the phosphates act somewhat in contrast to the nitrates and sulfates. In general the cation is negatively absorbed, while the anion is positively absorbed. This would seem to point to a splitting of the molecule were it not for the fact that we are dealing with a fraction of a milligram. The higher concentrations of calcium acid phosphate give negative adsorption for both ions. This may have been caused by a slight precipitation as a saturated solution was used.

Table 3 gives us the results obtained when the phosphates of calcium, magnesium and potassium were used with the hydrosol of silica. This hydrosol contained 3.1 per cent of silica. The columns are arranged similarly to those in the preceding tables.

Column 6 shows that the metals are always negatively adsorbed except in one case, and in general the negative adsorption is quite great. The phosphat ion, too, is negatively adsorbed in all but two cases, but the negative adsorption is not so great as that of the positive ion. This would indicate the same tendency in the splitting of the molecule as in the case of the hydrogel.

The tendency by which phosphates are held by the hydrogel is also interesting. Two samples of the hydrogel were shaken with 0.05 N solutions of potassium acid phosphate and calcium acid phosphate, respectively. All the solution was then drained from the gel which was placed on a filter and washed repeatedly with distilled water. The water was poured on the gel in 50-cc. portions and the gel allowed to drain thoroughly after each addition. Fifty-cubic centimeter portions of the washings were analyzed for phosphorus at intervals. Results are given in table 4.

It will be seen from table 4 that the salt that was held by the silica was remarkably resistant to washing. Also, that the portion of water obtained by washing after the gel stood overnight in a moist condition on the filter is much richer in phosphorus than washings obtained from the gel which stood for only a few minutes in a moist condition. A sample of the hydrogel which had been shaken with magnesium acid phosphate solution was washed six times a day for thirty days with 50-cc. portions of distilled water and at the end of that time still contained phosphate. The sample of hydrogel, washed

TABLE 1

Adsorption of nitrates and sulfates by the hydrosol of silica

1	2	3*	4	5	6	7	8	9	10	11
SOLUTIONS USED	METAL IN 20 CC. OF ORIGINAL SOLUTION	METAL IN 20 CC. OF SOLUTION AFTER DILUTION	METAL FOUND IN 20 CC. AFTER SHAKING	METAL ADSORBED PER GM. OF WET GEL	METAL ADSORBED PER GM. OF DRY GEL	ANION IN 20 CC. OF ORIGINAL SOLUTION	ANION IN 20 CC. AFTER DILUTION	ANION IN 20 CC. AFTER SHAKING	ANION ADSORBED PER GM. OF WET GEL	ANION ADSORBED PER GM. OF DRY GEL
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*		*mgm.*	*mgm.*	*mgm.*
KNO_3	18.096	15.994	16.048	0.20	−0.07	6.027	5.197	5.595	0.04	−0.06
$Ca(NO_3)_2$	12.420	11.660	12.290	0.02	−0.16	7.630	7.160	7.742	0.02	−0.15
$Mg(NO_3)_2$	12.510	11.530	10.931	0.24	0.02	14.552	13.410	12.530	0.03	0.29
K_2SO_4	39.248	31.420	36.480	0.42	−0.12	16.528	15.160	15.012	0.43	0.31
$CaSO_4$	3.880	3.543	3.665	0.03	−0.03	4.076	3.722	4.002	0.01	−0.06
$CaSO_4$	12.888	11.770	12.127	0.10	−0.09	9.066	8.260	8.919	0.02	−0.01
$MgSO_4$	12.349	11.428	11.205	0.17	0.07	15.910	14.666	15.189	0.01	−0.01

* The third column shows the theoretical weight of metal in 20 cc. of the solution provided all the water in the gel acted as a diluent. The fourth column indicates milligrams of metal actually found in 20 cc. after adsorption was completed. The fifth column gives the miligrams of metal adsorbed per gram of gel if water contained in the gel is not figured as diluting the solution upon which the adsorption test is made. The sixth column shows milligrams of metal absorbed if the water contained in the gel is figured as diluting the solution from which the adsorption test is made. From what has been said of the first six columns, columns seven to eleven, inclusive, should be clear.

TABLE 2

Adsorption of phosphates by hydrogel of salica

1	2	3	4	5	6	7	8	9	10	11
SOLUTIONS USED	METAL IN 10 CC. OF ORIGINAL SOLUTION	METAL IN 10 CC. AFTER DILUTION	METAL IN 10 CC. AFTER SHAKING	METAL TAKEN UP PER GM. WET GEL	METAL ADSORBED PER GM. DRY GEL	PO_4 IN 10 CC. OF ORIGINAL SOLUTION	PO_4 IN 10 CC. AFTER DILUTION	PO_4 IN 10 CC. AFTER SHAKING	PO_4 TAKEN UP PER GM. WET GEL	PO_4 TAKEN UP PER GM. DRY GEL
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*
$Ca(H_2PO_4)_2$	5.468*	4.510	5.070	1.19	−1.07	9.252	7.792	8.998	0.77	−2.29
	5.432*	4.837	5.040	......	−0.43	9.252	8.243	8.998	0.47	−1.60
	5.367	4.868	4.923	0.19	−0.01	9.602	8.700	8.505	0.32	0.06
	2.599	2.235	2.429	0.05	−0.03	4.917	4.456	4.370	0.16	0.03
	2.689†	2.436	2.282	0.06	0.03	4.012	4.451	4.385	0.07	0.01
	0.847‡	0.768	0.664	0.09	0.02	1.876	1.700	1.264	0.05	0.05
	5.740	5.140	5.740	0.00	−0.12	10.334	9.352	8.928	0.43	0.08
	2.820	2.520	3.164	−0.05	−0.12	5.246	4.747	4.536	0.22	0.04
	1.456	1.317	1.876	−0.06	−0.11	2.696	2.240	1.922	0.23	0.10
$Mg(H_2PO_4)_2$	5.140	4.960	5.140	0.00	−0.06	14.217	13.730	13.808	0.12	−0.01
	2.700	2.670	2.680	0.00	−0.00	7.497	7.243	7.229	0.22	0.00
	1.350	1.300	1.200	0.04	0.03	3.532	3.413	3.313	0.06	0.03
KH_2PO_4	12.600	11.500	11.200	2.15	0.05	10.545	9.810	8.783	0.27	0.25
	6.456	5.819	5.840	0.09	−0.01	5.270	4.890	4.003	0.19	0.25
	3.420	3.120	3.230	0.02	−0.20	2.830	2.630	2.091	0.11	0.13

* Amount in 1 cc.

† Amount in 20 cc.

‡ Amount in 50 cc.

TABLE 3

Adsorption of phosphates by hydrosol of silica

1	2	3	4	5	6	7	8	9	10	11
SOLUTIONS USED	METAL IN 10 CC. OF ORIGINAL SOLUTION	METAL IN 10 CC. AFTER DILUTION	METAL IN 10 CC. AFTER SHAKING	METAL ADSORBED PER GM. WET GEL	METAL ADSORBED PER GM. DRY GEL	PO$_4$ IN 10 CC. OF ORIGINAL SOLUTION	PO$_4$ IN 10 CC. AFTER DILUTION	PO$_4$ IN 10 CC. AFTER SHAKING	PO$_4$ ADSORBED PER GM. WET GEL	PO$_4$ ADSORBED PER GM. DRY GEL
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
$Ca(H_2PO_4)_2$	5.740	3.860	3.920	23.00	−0.65	10.334	6.950	7.171	45.00	−2.35
	2.820	1.900	2.128	9.90	−2.42	5.236	3.357	3.567	23.90	−2.23
	1.456	0.979	1.494	−0.04	−5.36	2.698	1.815	1.922	11.05	−1.18
$Mg(H_2PO_4)_2$	5.140	3.470	4.130	14.40	−12.41	14.217	11.436	10.632	51.20	8.49
	2.700	2.252	2.350	2.25	−4.97	7.970	6.031	6.110	26.60	−0.84
	1.350	1.126	1.356	0.08	−4.08	3.532	2.841	2.820	10.17	0.21
KH_2PO_4	12.680	8.060	8.760	56.06	−7.00	10.545	7.096	7.102	49.20	−0.06
	6.556	4.344	4.144	33.03	2.97	5.272	3.548	3.624	23.83	−0.80
	3.416	2.229	2.424	14.03	−1.32	2.830	1.904	2.375	6.50	−4.00

as described above, was placed in a funnel of such size that the 50-cc. portion of wash water covered it about 2 cm. deep. It is evident that a very great amount of rainfall would be necessary to duplicate the amount of washing given this sample, and that it would take a very great time for ordinary rainfall to wash the sample free from the salt.

TABLE 4

Phosphates found in 50-cc. portions of wash water

	PO_4 LEACHED FROM 1 GM. GEL.	
	Calcium acid phosphate solution	Potasssium acid phosphate solution
	mgm.	*mgm.*
First washing	4.15	4.74
Eleventh washing	0.10	0.14
Twenty first washing	0.07	0.13
Thirty first washing	0.06	0.12
Forty first washing	0.06	0.12
Forty second washing after standing over night	1.31	0.54

DISCUSSION

In reviewing the tables it must be remembered that we are dealing with milligrams in our work, and since the adsorptions are in general small and sometimes only .01 mgm., a slight error would change the order of the adsorption. On the other hand, the results seem to point to the fact that the metals and also the nitrates and sulfate radicals have a tendency to be negatively adsorbed, or not adsorbed at all by colloidal silica, while the phosphate radical tends to suffer a slight positive adsorption in the case of the hydrogel.

SUMMARY

1. The metals are, in general, negatively adsorbed by both the hydrogel and the hydrosol.
2. The nitrate and sulfate radicals of calcium, magnesium and potassium suffer, in general, a slight negative adsorption.
3. The phosphate radical was positively adsorbed by the hydrogel and negatively adsorbed by the hydrosol.
4. In the case of the hydrogel, the phosphate radical was adsorbed to a greater degree than the radical with which it was associated.
5. A phosphate adsorbed in the hydrogel can be washed only with extreme difficulty.

REFERENCES

(1) BIEDERMAN, R. 1869 Einige Beiträge zur der Frage der Bodenadsorption In Landw. Vers. Stat., v. 11, p. 1–95.

(2) FREY, J. 1875 Unterschungen über das Adsorptionsvermögen der Ackererde. *In* Landw. Vers. Stat., v. 18, p. 3–18.

(3) GORDON, NEIL E. 1922 Origin of soil colloids. *In* Science, v. 55, p. 676–677.

(4) GORDON, NEIL E., AND STARKEY, E. B. 1922 Influence of soil colloids on availability of salts. *In* Soil Sci., v. 14, p. 1–7.

(5) MATHIEU, J. 1902 Über die Capillarität der Lösungen. In Ann. Physik. (Leipzig), v. 9, p. 340.

(6) MOORE, C. J., FREY, W. H., AND MIDDLETON, H. E. 1921 Methods for determining the amount of colloidal material in soils. *In* Jour. Indus. Engin. Chem., v. 13, p. 527–530.

(7) SALOMON, ALEXANDER 1867 Versuche über die Adsorption des Kalkes. *In* Landw. Vers. Stat., v. 9, 351–355.

(8) VAN BEMMELEN, J. M. 1888 Die Adsorptionverbundungen und das Adsorptionsvermögen der Ackererde. *In* Landw. Vers. Stat., v. 35, p. 69–121.

INFLUENCE OF HYDROGEN-ION CONCENTRATION ON THE ADSORPTION OF PLANT FOOD BY SOIL COLLOIDS

E. B. STARKEY AND NEIL E. GORDON

University of Maryland

Received for publication July 13, 1922

INTRODUCTION

All know, according to Faraday's researches, that charges accumulate at the surface and, hence, at a boundary between two liquids, two solids, or liquid and solid, and that even between a gas and liquid we have a seat of electrical energy. Some believe that this seat has an electronic origin, while others believe that it is due to the Helmholtz's "double layer." In view of the latter, the charge on the surface of a solid or liquid would imply the existence of an equal and opposite charge on the other side of the interface, for a charge of a particular sign cannot exist without one of opposite sign in its proximity.

Perrin (6) has shown beyond the shadow of a doubt that the electrical charge on surfaces is in reality diminished, neutralized, or even reversed by ions of opposite charge. Acids and alakalies are very active in this power of conferring the electrical charges on surfaces. This is attributed to the fact that the H and OH ions are more readily absorbed than other ions, and, hence, the charge on an absorbent would depend on which of these ions is in excess.

Knud Estrup (4) has done some work along the line just mentioned. He showed that both the cation and anion were absorbed by charcoal in equivalent quantities when he worked with neutral salt solutions, and when the ions were not absorbed in equivalent quantities he attributed the difference to the presence of impurities in the form of acids and bases. In other work (3) with blood charcoal Estrup finds that the negative ion is positively absorbed in acid solutions, but on addition of a base the absorption of the negative ion disappears, giving place to the absorption of the positive ion.

It has been further shown that electrical charges tend to reduce surface tension. V. Boumat (1) finds that acids decrease surface tension and ascribes the fact to the accumulation of H-ions in the surface layer.

From the point of energetics, the following view of electrical adsorption has been formulated: Any process that will reduce the electrical energy at a surface will tend to take place, hence, in the case of a negatively charged surface, we will have positive charges collecting upon it in order to annul its charge, and vice versa. These bodies may be ions or colloidal aggregates.

A great many more or less obscure phenomena in the field of soil chemistry as well as in many other fields have been attributed to adsorption where it was not known whether they had physical adsorption, chemical reaction or an adsorption phenomena which might be attributed to charged aggregates where the adsorbing aggregate had a sign opposite to that of adsorbed substance.

Since it was known that OH and H ions were the most strongly adsorbed ions, and their adsorption should help to determine the charge on the colloid, it became of interest to determine the adsorption by single soil colloids under varying hydrogen-ion concentrations. It was hoped that such work might throw some definite light on how acidity or alkalinity of soil effects adsorption of plant foods, and also the availability of soil salts. A few of the results have already been published in a preliminary paper by Gordon and Starkey (5).

MATERIAL

The salts used were those prepared by the J. T. Baker Company and recommended by the National Research Council. They were the acid phosphates, sulfates and nitrates of potassium and calcium.

The hydrogels used were the hydrogels of silica and iron. The hydrogel of silica was obtained from the Davison Chemical Company. This hydrogel was washed free from all impurities before being used. The hydrogel of iron was prepared in our laboratory by precipitation from ferric chloride solution with ammonium hydroxide and washed free of ammonia and chlorides.

The apparatus used for determining the pH values of the solutions was of the Leeds, Northrup type. The Bailey hydrogen electrode was used in conjunction with a 1 *N* solution of KCl and the calomel electrode as described by Clarke (2). The mixtures of solutions and gels were shaken in 250-cc. ground-glass stoppered bottles with a mechanical shaker making about three oscillations per second.

TABLE 1

Adsorption of KH_2PO_4 by hydrogel of silica

NUMBER OF SOLUTION	REACTION	K ADSORBED PER GRAM GEL		PO_4 ADSORBED PER GRAM GEL	
		Dry	Wet	Dry	Wet
	pH	*mgm*	*mgm.*	*mgm.*	*mgm.*
1	9.501	9.63	9.99	−4.15	−0.57
2	7.692	6.56	7.40	−4.15	−0.57
3	6.086	1.74	2.18	−3.15	0.00
4	3.888	−0.68	0.71	−1.36	1.92

EXPERIMENTAL WORK

A series of four or five solutions of each of the salts used was made up. The pH values in each series were varied by adding the required amounts of NaOH or HCl (ranging from neutral to N/5) before the solutions were made up to the mark. The salt concentration was kept constant throughout. The concentration of each solution was determined by official methods.

In determining the adsorption of the various ions by hydrogel of silica, 200 cc. of each of the solutions of the salts was mixed with 50 gm. of the wet hydrogel of silica which had first been washed as free as possible of sulfates and which contained 43.7 per cent moisture. These mixtures were placed in 250-cc. ground-glass stoppered bottles and shaken for 72 hours. At the end of this time it was found that equilibrium had been reached. The mixtures were then allowed to settle until clear or, if a hydrosol was produced which would not settle, a Pasteur filter was used. Aliquots were then pipetted from the clear, supernatant liquid or filtrate and analyzed by the same methods as before the hydrogel had been mixed. The pH value of each of the solutions was also determined.

In the case of the hydrogel of iron, the same procedure was followed with the exception that 25 gm. of the wet hydrogel containing 95.5 per cent moisture, was used.

EXPERIMENTAL RESULTS

In the following tables, adsorption is indicated in milligrams adsorbed per gram of dry hydrogel. The negative sign denotes negative adsorption. All others are positive.

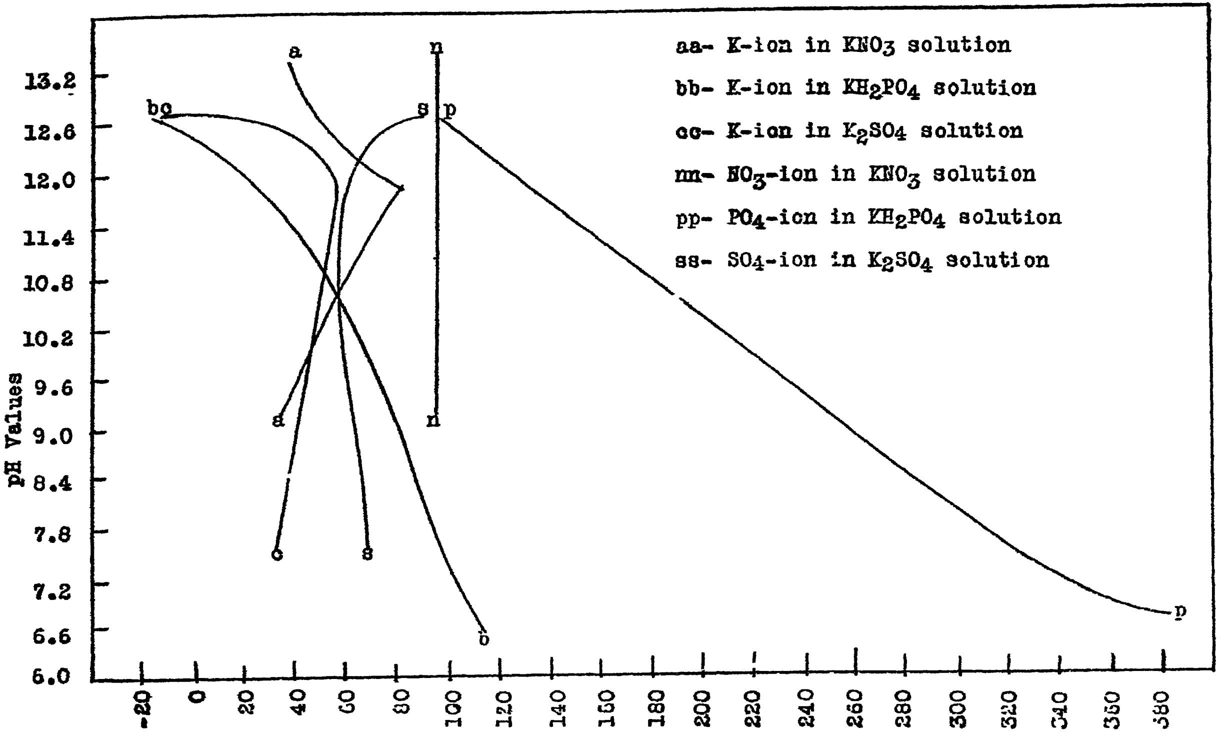
TABLE 2

Adsorption of potassium by the hydrogel of silica

NUMBER OF SOLUTION	KNO_3			K_2SO_4		
	Reaction	K ADSORBED PER GRAM GEL		Reaction	K ADSORBED PER GRAM GEL	
		Dry	Wet		Dry	Wet
	pH	*mgm.*	*mgm.*	*pH*	*mgm.*	*mgm.*
1	9.873	11.2	11.45	9.890	10.06	10.38
2	8.554	9.86	10.22	8.706	9.93	10.19
3	6.830	2.60	3.68	7.337	4.70	5.37
4	6.390	−1.07	0 37	7.117	1.91	2.79
5	3.360	−1.332	0.114	5.275	1.16	2.36

Results shown in the "dry" column were calculated not on a volume of 200 cc. of the solution, but on 200 cc. plus the amount of moisture contained in the hydrogel at the time it was added. Results shown in the "wet" column were calculated on a basis of 200 cc., the amount of the salt solution added. It will be noted that the amount of the positive potassium ion adsorbed decreased with a decrease of pH or OH concentration, showing the greatest adsorption took place in an alkaline solution, while the negative phosphate ion was adsorbed to a greater extent at high hydrogen-ion concentrations. This may be seen more plainly by referring to the curves on figure 1 of the authors' previous paper (4).

The adsorption of the potassium varied with the hydrogen-ion concentration as in table 1 where potassium acid phosphate was used. The curves in the above mentioned figure show how similar the adsorption of the potassium with the three salts was. Near the neutral point a small change in the pH value made a marked change in the amount of potassium adsorbed.



Mgm Adsorbed per gm Gel

FIG. 1. ADSORPTION OF K, NO_3, PO_4, SO_4

Table 3 gives the adsorption by hydrogel of silica in solutions of $CaH_4(PO_4)_2$. In the case of calcium salts it was impossible to obtain a wide range of pH values due to the precipitation of $Ca(OH)_2$ by NaOH. In table 3 it will be noted that solutions 2 and 3 had the same pH value. Solution 2 contained a small amount of NaOH and HCl in equal quantities, while solution 3 contained only the salt $CaH_4(PO_4)_2$.

The calcium ion was adsorbed in the same order as the potassium ion in the potassium salts, but the order of adsorption of the phosphate ion in this case was reversed over that in the potassium phosphate.

TABLE 3

Adsorption of $CaH_4\ (PO_4)_2$ by the hydrogel of silica

NUMBER OF SOLUTION	REACTION	Ca ADSORBED PER GRAM GEL		PO_4 ADSORBED PER GRAM GEL	
		Dry	Wet	Dry	Wet
	pH	*mgm*	*mgm.*	*mgm.*	*mgm.*
1	4.243	0.45	1.09	−0.64	2.56
2	4.193	0.41	1.05	−0.75	2.47
3	4.193	0.36	1.01	−1.00	1.87
4	4.142	0.18	0.85	−1.56	1.72
5	2.463	−0.89	−0.12	−2.62	0.77

TABLE 4

Adsorption of $Ca(NO_3)_2$ by the hydrogel of silica

NUMBER OF SOLUTION	REACTION	Ca ADSORBED PER GRAM GEL		NO_3 ADSORBED PER GRAM GEL	
		Dry	Wet	Dry	Wet
	pH	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1	6.695	0.61	1.25	−3.32	−0.88
2	6.120	−0.86	−0.07	−3.32	−0.88
3	6.086	−1.49	−0.64	−0.86	−1.33
4	6.052	−1.57	−0.71	−1.35	0.88
5	2.284	−2.86	−1.52	−1.35	0.88

The adsorption of calcium followed the same order as potassium, but the adsorption of the nitrate ion did not seem to be influenced to any great extent by the hydrogen-ion concentration though the general tendency was to decrease with a decrease in hydrogen-ion concentration. The maximum adsorption occurred in the solution which contained neither NaOH nor HCl. Solutions 1 and 2 contain .002 *N* and .0002 *N* NaOH, solution 3 contained only the salt, and solutions 4 and 5 contain .002 *N* and .0002 *N* HCl. It would appear that the presence of these compounds influenced the adsorption of the nitrate ion.

Tables 5, 6, 7, 8, and 9 give the adsorption of salts by the hydrogel of ferric hydroxide. The pH values in the case of this gel were made much higher than the corresponding pH values for the silica gel, in order to avoid a chemical solution with the hydrogel.

Table 5 shows that the negative phosphate ion is adsorbed to the greatest extent in the lower hydroxyl-ion concentration and decreases as the hydroxyl-ion concentration increases. But the positive potassium-ion does not follow the general rule of the positive ion. This is the only case found so far where the positive ion adsorption increases with the decrease of pH values throughout.

TABLE 5

Adsorption of KH_2PO_4 by the hydrogel of ferric hydroxide

NUMBER OF SOLUTION	REACTION	K ADSORBED PER GRAM GEL		PO_4 ADSORBED PER GRAM GEL	
		Dry	Wet	Dry	Wet
	pH	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1	12.730	−65.2	−16.3	9.3	97.2
2	11.868	−14.3	29.2	51.2	135.0
3	7.439	63.0	99.0	216.0	324.1
4	6.695	77.7	111.2	320.9	378.1
5	6.627	80.9	114.1	329.9	386.2

TABLE 6

Adsorption of KNO_3 by the hydrogel of ferric hydroxide

NUMBER OF SOLUTION	REACTION	K ADSORBED PER GRAM GEL		NO_3 ADSORBED PER GRAM GEL	
		Dry	Wet	Dry	Wet
	pH	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1	13.389	−0.5	39.9	34.4	97.8
2	12.612	11.0	50.2	34.4	97.8
3	11.868	48.9	84.0	34.4	97.8
4	9.417	0.2	40.4	34.4	97.8
5	9.281	−4.9	36.2	34.4	97.8

From table 6 it is seen that the adsorption of the anion was not affected by a change of pH values but that its adsorption rate was independent of other ions present. The cation seemed to present a maximum adsorption in this case around a pH value of 11.868. The same will be noticed in table 7 which shows a minimum adsorption of the sulfate ion at the same pH value. Curves in figure 1 make this more plain.

The maximum adsorption of the potassium ion at a pH value of 11.530, and the minimum adsorption of the sulfate ion at the same hydrogen-ion concentration is worthy of note. This is better shown by the curves in figure 1.

In table 8 we find the greatest adsorption throughout that has been noticed so far. While the change of pH values seems to have had but little effect on the adsorption of the phosphate ion, it has a tendency to present a minimum adsorption as was noted in table 7. The cation here followed the general order of cation adsorption with a change of pH value.

It will be seen in table 9 that there was slightly greater adsorption in solution 5 than in solution 4, which breaks the order of the cation adsorption. This is accounted for by the fact that solution 5 suffered such a high peptization, that the accurate determination of the adsorption became very difficult. Here again we notice the general tendency of the nitrate ion not to be affected by the pH value.

TABLE 7

Adsorption of K_2SO_4 by the hydrogel of ferric hydroxide

NUMBER OF SOLUTION	REACTION	K ADSORBED PER GRAM GEL		SO_4 ADSORBED PER GRAM GEL	
		Dry	Wet	Dry	Wet
	pH	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1	12.645	−60.8	−14.5	44.2	91.1
2	12.578	−01.0	38.9	21.2	70.5
3	11.530	21.7	59.2	10.1	60.7
4	8.182	−02.4	37.7	19.1	68.9
5	7.624	−07.8	32.8	19.1	68.9

TABLE 8

Adsorption of $CaH_4(PO_4)_2$ by the hydrogel of ferric hydroxide

NUMBER OF SOLUTION	REACTION	Ca ADSORBED PER GRAM GEL		PO_4 ADSORBED PER GRAM GEL	
		Dry	Wet	Dry	Wet
	pH	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1	5.156	109.5	118.6	554.8	589.0
2	5.156	104.5	114.1	536.8	572.8
3	5.038	101.9	111.8	536.8	572.8
4	5.021	101.9	111.8	581.7	613.3
5	4.446	56.4	71.2	587.7	618.7

TABLE 9

Adsorption of $Ca(NO_3)_2$ by the hydrogel of ferric hydroxide

NUMBER OF SOLUTION	REACTION	Ca ADSORBED PER GRAM GEL		NO_3 ADSORBED PER GRAM GEL	
		Dry	Wet	Dry	Wet
	pH	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1	6.036	29.3	47.6	−12.0	62.0
2	5.241	−03.6	18.3	−12.0	62.0
3	5.122	−39.1	−13.3	−12.0	62.0
4	5.038	−46.4	−20.0	−12.0	62.0
5	4.432	−31.5	−06.5	−12.0	62.0

Inasmuch as it seems to be the general tendency for an acid reaction to fasten the negative ion and an alkali reaction to fasten the positive ion, we believed that by reversing the charge on the gel we should get a release of the ions which were adsorbed the most strongly. That is to say, by washing a gel which had adsorbed a negative ion strongly with an alkali, the ion should be released or washed out. So we proceeded in the following manner.

The silica gels from solutions 1, 2, 4 and 5 of table 1 (Absorption of KH_2PO_4 by hydrogel of *silica*) were used. Solutions 1 and 2 contained or had absorbed a relatively large amount of the potassium while solutions 4 and 5 had absorbed the phosphate ion. All the samples were then placed in filter papers in separate funnels and washed with water to remove that portion of solution mechanically held. Analysis of these portions were made as shown in the results. After the analysis showed only a small quantity of potassium and phosphate ions in the wash water, the acid and alkali washings were begun. For this, $0.1N$ HCl or NaOH were added in 50-cc portions. The filtrates were collected in separate beakers. To gel 1, 1 N HCl was added; to gel 2, $0.1N$ NaOH; to gel 4, $0.1N$ HCl and to gel 5, $1.0N$ NaOH. Three successive samples of washings were collected from each of the gels, then a fourth portion was added to each and allowed to stand over night. This was run off the next morning and analyzed along with the others. Table 10 gives the results obtained from the analyses of the HCl and corresponding previous water washings.

TABLE 10

Analysis of HCl washings of hydrogel of silica

WASHING	GEL NO. 1 K RELEASED	GEL NO. 4 PO_4 RELEASED
	mgm.	*mgm.*
1st 50 cc. H_2O	15.52	85.31
3rd 50 cc. H_2O	0.83	7.33
1st 50 cc. HCl	22.91	5.19
2nd 50 cc. HCl	21.96	2.70
3rd 50 cc. HCl	19.84	1.63
Over night HCl	24.37	8.69

TABLE 11

Analysis of NaOH washings of hydrogel of silica

WASHING	GEL NO. 2 K RELEASED	GEL NO. 5 PO_4 RELEASED
	mgm.	*mgm.*
1st 50 cc. H_2O	20.67	95.65
3rd 50 cc. H_2O	1.66	1.09
1st 50 cc. NaOH	2.94	2.70
2nd 50 cc. NaOH	3.65	1.35
3rd 50 cc. NaOH	2.59	1.09
Over night NaOH	14.77	7.88

Table 11 gives the results obtained from the analyses of the NaOH and corresponding water washings.

It will be seen from table 10 that the HCl washing caused a marked release of the potassium ion, whereas no increase was noticed in the phosphate ion until the HCl was left overnight in contact with the gel.

In table 11 it will be noticed that the NaOH caused a slight increase in the liberation or movement of the phosphate ion. The small increase was probably due to the small amount present to begin with. We also obtained a slightly increased movement of the potassium ion. This is accounted for by a replacement of the potassium by the sodium ion until a state of equilibrium is reached. The explanation becomes even more plausible when the results for the overnight washings are observed.

DISCUSSION

We had expected when starting the work that an increase in the concentration of the hydroxyl ions would increase the adsorption of cations by soil colloids. These results were very generally found as seen in the preceding tables. This was anticipated in accordance with Helmholtz's electrical double-layer theory which assumes that one ion may become more closely associated with the colloidal particle than the other ion. This most closely associated ion would impart its charge to the colloidal particle, but this charged colloid and ion will attract ions of opposite charge and in our case, if it is the OH ion that is giving the charge to the colloid, it should attract the metal ions which have the opposite sign. Hence, the solution should become poorer in metallic ions. This was what was found, but this is only one explanation of the general behavior of the metallic ions. The negative ions showed many specific cases, and work is now in progress to explain the entire phenomenon in terms of chemical reactions which are governed by difference of solubility or hydrogen-ion concentration.

SUMMARY

1. The adsorption of the cation as a rule increased with increased hydroxyl ion concentration, or with increased pH value.
2. The adsorption of the phosphate ion increased with decreased pH value in some cases.
3. Adsorption of the nitrate and sulfate ions was not consistently influenced by the reaction of the solutions.
4. Anions seem to be adsorbed in the order of phosphate, sulfate and nitrate with phosphate well in the lead.
5. Adsorption by ferric hydroxide gel is greater than by silicic acid gel.
6. There is a large change in adsorption of potassium ions for very slight change in the hydrogen-ion concentration around the neutral point.

REFERENCES

(1) BOURNAT, M. V. 1909 Adsorption D'ions. *In* Compt. Rend. Acad. Sci. (Paris), v. 149, p. 1366-1368.

(2) CLARKE, W. M. 1920 The Determination of Hydrogen Ions, p. 133-141. Williams & Wilkins Company, Baltimore, Md.

(3) ESTRUP, K. 1912 Studies of negative adsorption. *In* Overs. K. Danske Vidensk. Selsk. Forhandl., Nr 4, p. 238-259.

(4) ESTRUP, K. 1913 Studies in electrolytic adsorption. *In* Overs. K. Danske Vidensk Selsk. Forhandl., Nr 1, p. 14-45.

(5) GORDON, NEIL E., AND STARKEY, E. B. 1922 Influence of soil colloids on availability of salts. *In* Soil Sci., v. 14, p. 1-7.

(6) PERRIN, JEAN B. 1904 Mécanism de l'electrisation de contact et solutions colloidales. *In* Jour. de Chim. Phys., v. 2, p. 601.

OXIDATION OF ZINC SULFIDE BY MICROÖRGANISMS[1]

W. RUDOLFS AND ANDRÉ HELBRONNER

New Jersey Agricultural Experiment Stations

Received for publication July 1, 1922

Under conditions of natural exposure, hydrogen sulfide and metallic sulfides are slowly oxidized. In a fine state of subdivision, and in the presence of catalytic agents oxidation proceeds and sulfuric acid is formed. The sulfuric acid in turn reacts with the bases to form sulfates. Winogradksy (10, 11) described a group of sulfur bacteria which were able to reduce H_2S to elementary sulfur, and to oxidize elementary sulfur to H_2SO_4. The sulfur transformation is given in the following reactions:

$$2\ H_2S + O_2 = 2\ H_2O + S_2 + 122 \text{ calories}$$
$$S_2 + 3\ O_2 + 2\ H_2O = 2\ H_2SO_4 + 282 \text{ calories}$$

While sulfur oxidation in soils appears to be chiefly a biological phenomenon, there seems no doubt that the results are influenced by purely chemical reactions.

Kappen and Quensell (4), Van Bemmelen (9) and MacIntire and his associates (6) conclude that sulfur oxidation takes place without the aid of bacteria. Boulanger (1), Demolon (2), Lipman and his assistants (5) have laid much emphasis on the biological factor in sulfur oxidation in the soil. Lipman seized upon the idea of utilizing this factor for agricultural purposes. Rock phosphate was mixed with flowers of sulfur, and the mixture inoculated with sulfur-oxidizing organisms. The sulfur, transformed into H_2SO_4, reacts with the $Ca_3(PO_4)_2$ rendering the inert phosphates soluble and available to plants.

Little is known about the influence of microorganisms upon the oxidation of metallic sulfides. Rudolfs (7) found that certain sulfur-oxidizing bacteria readily oxidize pyrites. As far as the writers were able to learn, nothing else is on record as to possible biological reactions involving metallic sulfides.

PROCEDURE

With a mixed culture of a large group of sulfur-oxidizing organisms we finally succeeded in securing a culture which was able to slowly attack precipitated ZnS. From these cultures the best was selected, inoculated into a sterilized soil medium, and mixed with a nearly pure Spanish zinc blende. This culture served as inoculum in mixtures of unsterilized soil with two commercial zinc sulfides. The cultures were made by thoroughly mixing air-dry portions of soil, blende, and in some cases flowers of sulfur. The soil used was Penn loam. Inoculations were made with a water extract of the original culture in portions of the new material. The mixtures consisted of:

---

[1] Paper No. 95 of the Journal Series, New Jersey Agricultural Experiment Stations, Department of Soil Chemistry and Bacteriology.

This paper will appear in Rutgers College Studies, vol. 1.

NUMBER	SOIL	ORE	SULFUR	INOCULATION
	gm.	*gm.*	*gm.*	
1	250	75	None	None
2	250	75	None	Soil
3	250	75	10	Sulfur
4	245	75	10	Blende
5	240	75	25	Soil

Triplicate composts were placed in tumblers covered with glass plates and incubated at 28°C. Each culture was weighed and the water-holding capacity determined. Samples were weighed out and relative acidities, hydrogen-ion concentrations, sulfates and soluble zinc determined. The composts were kept at approximately 60 per cent of the water-holding capacity throughout the period of incubation. The amount of water lost by evaporation determined by placing the tumblers on the scale pan, was added each week and the mixtures removed from the tumblers to provide for sufficient aeration.

Water-extractions were made of weighed air-dry samples from each culture, by shaking 20 gm. of mixture with 200 cc. of diluted water in 1-liter flasks in a shaking machine for 2 hours. The flasks were left standing over night and an aliquot drawn off for the pH determinations. The remainder of the contents of the flasks were then filtered until the liquid was clear.

The hydrogen-ion concentrations were determined by the colorimetric method, with the apparatus described by Van Alstine (8).

TABLE 1

Analyses of zinc ores

ORE	Zn	Fe	CaO	S	Pb	SO_4	REACTION	MOISTURE
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*pH*	*per cent*
Zinc concentrates..............	46.6	3.9	4.9	29.2	0.12	0.037	7.4	0.14
Zinc blende....................	48.4	7.7	2.7	31.2	0.20	0.060	7.7	0.64
Penn loam......................		9.4	0.32	0.04		0.119	6.7	2.34

Determinations of relative acidity were made upon aliquots of the water extract. The liquid was boiled to expell CO_2, cooled, and titrated with 0.1 *N* NaOH, using phenolphthalein as indicator.

The sulfates were determined by acidifying aliquots of the water extract with concentrated HCl and precipitating at the boiling point with $BaCl_2$.

Soluble zinc was determined by the electrolytic method proposed by Classen (3) and by the volumetric method with ferrocyanide solution, using uranium acetate as indicator. Iron, etc., was removed before titration. The results recorded are calculated to the moisture-free basis of the soil and ores. The analyses of the ores and soil at the beginning of the investigation are given in table 1.

EXPERIMENTAL RESULTS

For a study of the progress of the reaction, details of two series of cultures are given in tables 2 and 3. The water-soluble acidity increased continuously, while the hydrogen-ion concentration fluctuated. In general the hydrogen-ion concentration shows a sudden increase, then a slow decrease, and again an increase. Determinations of pH values do not show the total acidity in the mixtures, but merely the strength of the free acid at the time the determinations were made. These determinations serve as a convenient means of following the process of oxidation and the general reaction. The sudden increase marks a rapid oxidation of sulfur to free H_2SO_4, which in turn reacts with the

TABLE 2

Reaction of mixtures after various periods of incubation

CULTURE NUMBER	INITIAL		1 WEEK	2 WEEKS	3 WEEKS		6 WEEKS		9 WEEKS		12 WEEKS		18 WEEKS	
	*cc.**	*pH*	*pH*	*pH*	*cc.*	*pH*	*cc.*	*pH*	*cc.*	*pH*	*cc.*	*pH*	*cc.*	*pH*
1	0.0	7.2	7.1	7.1	0.1	7.2	0.1	7.0	0.1	7.1	0.4	6.6	0.6	6.4
2	0.0	7.2	7.0	6.9	0.3	6.6	0.6	6.4	1.0	6.2	2.6	5.2	5.2	5.0
3	0.0	7.2	6.6	3.5	12.0	3.2	21.2	3.7	22.8	4.0	27.6	3.4	55.6	3.5
4	0.1	6.9	6.5	4.2	4.6	3.2	18.4	3.4	19.6	4.0	25.4	3.3	50.8	3.7
5	0.0	7.1	6.1	3.7	10.8	3.1	24.8	3.6	27.2	3.1	52.4	2.9	79.6	3.0
6	0.0	7.2	7.1	7.0	0.0	7.1	0.0	6.7	0.1	6.6	0.2	6.7	0.3	6.5
7	0.0	7.2	7.1	6.6	0.4	6.6	2.0	5.7	3.0	4.6	10.6	4.7	30.2	4.6
8	0.0	7.1	6.8	6.7	0.6	6.5	8.0	4.5	19.2	4.6	32.0	4.4	63.2	4.4
9	0.0	7.0	6.7	6.6	0.4	6.7	7.4	4.8	17.2	4.6	34.0	3.8	72.4	4.1
10	0.0	7.1	6.3	6.7	1.0	6.4	14.6	3.8	26.0	4.5	42.0	4.3	62.8	4.0

* Acidity expressed in cc. 0.1 *N* NaOH required to neutralize acidity of water extract from 10 gms. of mixture.

TABLE 3

Accumulation of water-soluble zinc and sulfate

Per 10 grams of mixture

CULTURE NUMBER	INITIAL		AFTER 1 WEEK		AFTER 3 WEEKS		AFTER 6 WEEKS		AFTER 9 WEEKS		AFTER 12 WEEKS		AFTER 18 WEEKS		PROPORTION OF TOTAL ZINC RENDERED SOLUBLE
	SO_4	Zn	SO_4	Zn	SO_4	Zn	SO_4	Zn	SO_4	Zn	SO_4	Zn	SO_4	Zn	
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*per cent*
1	5.0	3.2	5.4	2.9	7.0	3.1	13.8	7.9	29.0	12.6	33.4	16.4	50.6	18.0	1.67
2	5.1	3.7	13.4	6.4	24.2	12.2	58.2	34.7	102.1	51.4	114.2	63.0	142.2	98.0	8.32
3	10.1	3.6	17.2	12.8	146.4	90.6	198.9	134.5	256.2	157.4	282.0	172.6	377.8	190.0	19.17
4	11.1	3.8	18.1	19.2	133.1	77.7	182.8	149.4	186.4	150.6	228.8	180.0	364.2	192.5	17.59
5	18.8	3.7	35.6	18.2	121.8	64.4	202.1	191.1	220.1	204.0	358.3	297.2	518.1	369.0	37.56
6	5.4	3.1	5.4	3.3	7.6	4.1	21.9	15.8	43.8	30.4	81.8	30.6	139.2	67.5	5.81
7	5.4	3.2	6.8	5.6	37.9	6.2	53.0	17.6	62.9	40.7	141.3	81.0	271.8	193.3	17.65
8	19.1	3.0	41.9	6.2	151.5	102.9	195.1	159.8	258.7	166.2	327.0	195.2	488.0	255.0	23.82
9	14.2	3.1	19.3	6.2	138.4	89.1	196.8	101.5	253.4	123.3	342.0	194.4	545.8	270.0	25.42
10	18.7	3.1	27.9	6.1	137.4	93.1	211.6	159.8	301.8	191.5	393.1	198.2	505.0	245.0	23.76

basic constituents of the mixtures. The decrease of hydrogen-ion concentration indicates a more complete reaction. As may be seen from table 3, sulfur oxidation takes place in all cultures.

Apparently as the result of chemical reactions a small amount of zinc sulfide was converted into zinc sulfate. This agrees with the findings in sterilized cultures which had been incubated for 30 weeks. However, the inoculated cultures made far more zinc soluble than the uninoculated mixtures; while mixtures to which elementary sulfur was added showed still greater amounts of soluble zinc. This seemed to indicate that in mixtures containing high proportions of soluble zinc, sufficient sulfuric acid was produced by the sulfur-oxidizing organisms to react with the zinc ore.

TABLE 4

Soluble zinc from ores incubated for a period of 18 weeks

TREATMENT OF MIXTURE OF 250 GM SOIL AND 75 GM ORE	WILLEMITE	ZINC CARBONATE	LOW GRADE SILICATE ORE
	per cent	*per cent*	*per cent*
Uninoculated	0.41	1.02	2.10
5 gm. inoculated soil	0.23	1.14	2.06
10 gm. inoculated sulfur	15.53	16.03	74.91
5 gm. inoculated blende and 10 gm. sulfur	6.81	19.73	47.27
10 gm. inoculated soil and 25 gm. sulfur	9.99	22.67	68.44

A number of experiments were then made with other zinc ores, notably with Willemite (Zn_2SiO_4), Smithsonite ($ZnCO_3$) and a low silicate ore. The cultures were inoculated with a pure strain of sulfur-oxidizing organisms, known to convert sulfur into sulfuric acid and analyzed as described above.

The results at the end of 18 weeks are given in table 4.

It is evident from table 4 that the sulfuric acid produced by the oxidation of elementary sulfur readily transformed these different ores.

It is a point of interest to know at which hydrogen-ion concentration transformation of ZnS to $ZnSO_4$ takes place. To find the exact acidity necessary to change the different zinc ores, curves were constructed from readings of pH values obtained by additions of different amounts of 0.1 *N* H_2SO_4 to the ores. Ten grams of ore were shaken for 2 hours in a shaking machine with definite amounts of sulfuric acid added to distilled water to make 200 cc. of liquid. The bottles with contents were left standing for 24 hours and an aliquot of the supernatant liquid was then drawn off and the hydrogen-ion determinations made. At the critical points sufficient determinations were made to check up all points obtained. The results for one zinc sulfide ore are graphically shown in figure 1. It is apparent that at approximately pH 5.7 to 5.5 there occurred a sudden increase in the solubility of the ZnS, indicating that but very weak acidity is required to reach to critical point of solubility. However, with even weaker acidity a rapid reaction takes place. If this graph is compared with the figures in the tables, the relation between the hydrogen-ion concentration and $ZnSO_4$ formation is strikingly shown.

Since a possible practical application would necessarily be directed toward the low grade ores, the part of the work dealing with the low grade silicate ore was most interesting. However, an addition of elementary sulfur to such ores would considerably increase the cost of operation, and no profitable use could be made of this biological method.

Studies now under way may result in reducing the incubation period, which in the above tests had to be prolonged.

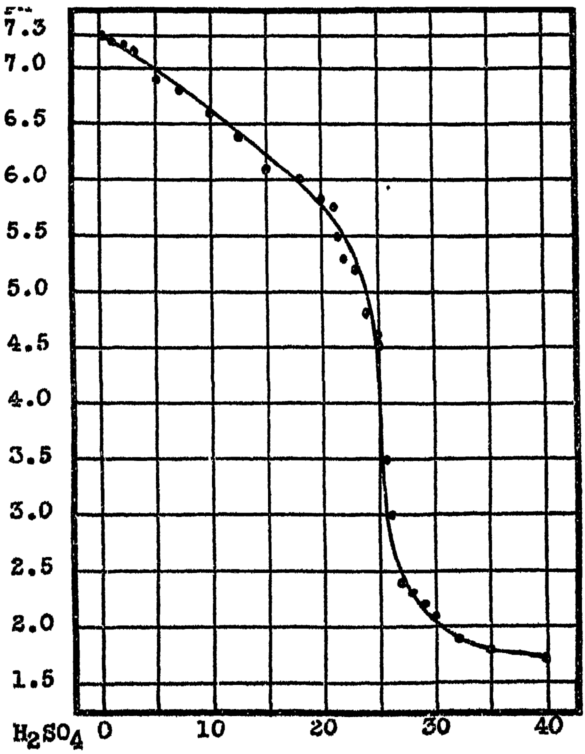


Fig. 1. Graph Showing at Which Hydrogen-Ion Concentration the Zinc of Zinc Sulfide Becomes Soluble

From a practical view point it seems more interesting to develop a strong oxidizing-culture which needs little or no free sulfur with which to start. This would limit the work to low-grade sulfides. Since enormous quantities of low-grade sulfide ores are found and are at present rarely used, this point was therefore investigated.

Low grade zinc ores are frequently associated with other sulfides, so much so, in fact, that economic geologists usually consider lead sulfide and zinc sulfide together.

A study was made with rather low-grade zinc sulfide containing equal amounts of zinc sulfide and of galena (PbS). Mixtures were made of 50 parts of soil, 50 parts of zinc sulfide (14.5 per cent Zn), and 50 parts of galena. Sulfur was added to some mixtures as to the cultures reported in table 1; uninoculated check cultures and mixtures with zinc sulfide and galena alone were included in the series. After 12 weeks, 24.5 per cent of the zinc was soluble in the cultures to which sulfur had been added, while 10.1 per cent zinc was soluble in the inoculated mixtures without sulfur. No trace of lead was present in any of the extracts. After 30 weeks, 72.4 per cent zinc was soluble in mixtures without sulfur and a trace of lead appeared.

From the data presented the following tentative conclusions may be drawn:

1. Microörganisms are able to transform zinc sulfide to zinc sulfate.
2. The growth of these organisms is not inhibited by the resulting soluble zinc.
3. The addition of elementary sulfur to impure cultures increases the rate of solubility of zinc blende.
4. The "Lipman" sulfur-oxidizing organisms produce sufficient H_2SO_4 from elementary sulfur to render zinc carbonate and zinc silicate soluble.
5. A possible biological method can be worked out for economical utilization of low-grade zinc sulfide ores.

Studies are under way aimed to reduce the amount of soil or to supplant it by a suitable medium; to determine the influence of temperature and moisture-content of the mixtures; and to purify the bacteria which use the combined sulfur as a source of energy. These studies will be reported on subsequently.

REFERENCES

(1) Boulanger, E. 1912 L'action du soufre en fleur sur la végétation. *In* Compt. Rend. Acad. Sci. (Paris), v. 154, p. 369–370.

(2) Demolon, H. 1913 Récherches sur l'action fertilisante du soufre. *In* Compt. Rend. Acad. Sci. (Paris), v. 156, p. 725–728.

(3) Classen, Alexander 1903 Quantitative Analysis by Electrolysis, p. 146. John Wiley, New York City.

(4) Kappen, H., and Quensell, E. 1915 Über die Umwandlungen von Schwefel und Schwefelverbindungen in Ackerboden. *In* Landw. Vers. Stat., v. 86, p. 1–34.

(5) Lipman, J. C., McLean H., and Lint, H. C. 1916 Sulfur oxidation in soils and its effect on the availability of mineral phosphates. *In* Soil. Sci., v. 2, p. 499–538.

(6) MacIntire, W. H., Gray, F. J., and Shaw, W. M. 1921 The non-biological oxidation of elemental sulfur in quartz media. *In* Soil Sci., v. 14, p. 135–147.

(7) Rudolfs, W. 1922 Oxidation of pyrites by microörganisms and their use for making mineral phosphates available. *In* Soil Sci., v. 14, p. 135–147.

(8) Van Alstine, E. 1920 The determination of hydrogen-ion concentration by the colorimetric method and an apparatus for rapid and accurate work. *In* Soil Sci., v. 10, p. 467–478.

(9) Van Bemmelen, J. 1886 Bijdragen tot de kennis van den Alluvialen bodem in Nederland. Holkema and Warendorf, Amsterdam.

(10) Winogradsky, S. 1887 Über Schwefelbakterien. *In* Bot. Ztg., v. 45, no. 31–37, p. 488–610.

(11) Winogradsky, S. 1888 Beiträge zur Morphologie und Physiologie der Bakterien. I. Schwefelbakterien, 1–120. A. Felix, Leipzig.

MODIFICATIONS OF THE SOIL FLORA INDUCED BY APPLICATIONS OF CRUDE PETROLEUM[1]

I. L. BALDWIN

Purdue University

Received for publication January 11, 1922

Although crude petroleum is not normally used as an antiseptic or germicide, it undoubtedly has a certain amount of germicidal action and its action in the soil is in all probability very largely that of an antiseptic.

In addition to this action, several investigators have shown that certain bacteria have the power of utilizing the hydrocarbons contained in crude petroleum as sources of carbon and energy. Crude petroleum therefore may modify the soil flora in much the same way as does large applications of carbohydrates, such as straw.

The literature contains very few references to the effect of crude petroleum on the soil flora and biochemical reactions of the soil. However a great deal of work has been carried out in studying the action of various other materials used as soil antiseptics. The materials most commonly used in this connection have been carbon disulfide, ether, toluene and benzene. While the data, with respect to the action of these substances, are largely conflicting, the majority of investigators have reported a temporary decrease in bacterial numbers and biochemical activities, later followed by a large increase.

Rahn (4) reported in 1906, the presence in soil of a mold belonging to the Penicillium group which was capable of breaking down paraffin and using it as a source of food.

Sohngen (5), a number of years later, gave an extensive report of a number of bacteria isolated from soil which are capable of using paraffin, petroleum and benzene as sources of carbon and energy.

Tausz and Peter (6), in an extensive report on methods of analysis of hydrocarbons, describe several organisms capable of utilizing definite hydrocarbons as sources of carbon and energy.

Gainey (2), in an article discussing the effect of paraffin on ammonification and nitrification in the soil, concludes that these biochemical activities are very materially hindered by the presence of this substance.

Carr (3), in making applications of crude petroleum to soil growing soy beans, found that their growth was apparently improved through the addition of small amounts of crude petroleum (up to 0.75 per cent), and that rather large amounts could be mixed with the soil (4.0 per cent) before it killed the soy bean plant.

---

[1] Part of a thesis submitted to the faculty of Purdue University in partial fulfillment of the requirements for the degree of Master of Science in Agriculture.

This opportunity is taken to express to Dr. R. H. Carr, due appreciation for his suggestions and criticisms of this work.

TECHNIC

Crude petroleum having a specific gravity of 0.8370 was used in all of the investigations made. The sample was secured from the Indiana Pipe Line Company at the Kankakee Pumping Station, just as it came from the pipe line, and was kept in a closed vessel at all times in order to avoid any outside contamination.

The methods of making the application of this crude petroleum to the soil varied with the type of experiment being conducted and will be described in detail in connection with the various experiments.

Bacterial counts of the soil samples were made by plating on agar. A number of different media recommended by various investigators were tried out in an effort to find the one which would prove the most satisfactory both from the standpoint of the number of colonies developing and the production of characteristic colonies. Although the media did not vary considerably in their degrees of suitability, it was finally decided that a synthetic medium of the following composition and adjusted to a hydrogen concentration of pH 7 was the most satisfactory.

Sodium potassium tartrate	5.0 gm.
Peptone	1.0 gm.
Dibasic potassium phosphate	0.5 gm.
Magnesium sulfate	0.2 gm.
Agar	15.0 gm.
Distilled water	1 liter

Ammonia was determined by direct distillation with magnesium oxide. It is recognized that this method of determination may give results which are slightly high, because of the breaking down of certain amides and amino acids into ammonia. The results are, however, comparable one with another if the same technic is used in all cases.

Nitrate determinations were made by the phenol disulfonic acid method, as modified by Noyes (3) for soil analysis. When the work was first started and the complete absence of nitrates was noted in the soil treated with the crude petroleum, it was thought that the presence of the crude petroleum might interfere with the colormetric determination of nitrates. To check this point, a known quantity of nitrate was added to a sample of soil containing a heavy application of the crude petroleum, and the nitrate was recovered quantitatively by the phenol disulfonic acid method. In addition, samples showing no nitrates by the phenol disulfonic acid method were also analysed for nitrates by the Devardo method and the same results were obtained. It is felt that the method used is very well adapted to the work, since extremely small amounts of nitrate may be detected.

Crude petroleum was determined in the soil by extraction with equal parts of petroleum and ethyl ethers. The material extracted was dried at a temperature of 37.5°C. for 24 hours and then placed over calcium chloride to complete the drying process. The drying was carried out in this manner to avoid any unnecessary loss of the more volatile portions from the crude petroleum. Blanks on untreated soil were determined in order to make correction for the material other than crude petroleum which might be extracted.

FIELD PLOT EXPERIMENTS

For this part of the work a healthy normal row was selected from an experimental corn plot. This corn received no treatment other than the application of crude petroleum. The soil was a dark silt loam containing about 7 per cent volatile matter, and the corn was of the yellow dent variety. The crude petroleum was applied in varying amounts to the individual hills of corn, after it was about 1 foot high. This application was made to the surface of the soil in a circle about the hill at a distance of about 6 inches from the plants. The row and the hills selected for this experiment were as representative and normal as it was possible to get them. Applications ranging from 25 cc. to 425 cc. were made to each hill.

Samples were taken from each of the treated hills at two different dates and moisture, nitrates, nitrification and bacterial counts determined.

Both aerobic and anaerobic bacterial counts were secured. The anaerobic count was secured by incubating the plates in an atmosphere of flowing hydrogen.

Nitrification was determined by setting 100 gm. of the soil away in a tumbler for a two weeks' incubation period. Nothing other than water to bring the moisture content to optimum was added to the soil.

TABLE 1

Field plot experiment

CRUDE PETROLEUM PER HILL	AUGUST 7, 1920				OCTOBER 16, 1920			
	Nitrates, on dry basis		Bacteria per gram of dry soil		Nitrates, on dry basis		Bacteria per gram of dry soil	
	Fresh samples	After 2 weeks incubation	Aerobes	Anaerobes	Fresh samples	After 2 weeks incubation	Aerobes	Anaerobes
cc.	*p.p.m.*	*p.p.m.*	*millions*	*millions*	*p.p.m.*	*p.p.m.*	*millions*	*millions*
0	22.41	56.30	1.67	0.178	26.74	73.20	2.81	0.184
25	2.52	16.80	2.52	0.173	13.42	19.92	2.84	0.192
75	1.17	5.50	3.25	0.135	6.78	16.81	3.02	0.178
125	0.33	3.37	3.38	0.176	4.78	14.35	3.35	0.164
175	0.00	1.67	2.88	0.209	1.79	3.42	3.21	0.192
225	0.00	1.67	3.28	0.201	0.00	1.68	3.78	0.135
275	0.00	1.68	3.92	0.209	0.00	1.68	3.91	0.186
325	0.00	1.67	4.69	0.194	0.00	1.67	4.73	0.203
375	0.00	1.25	4.78	0.197	0.00	1.25	4.53	0.211
425	0.00	0.75	5.24	0.203	0.00	1.25	6.78	0.203

By examination of table 1, it will be seen that the nitrate content of the fresh soil was materially lowered in both sets of samples. Also the quantity of nitrate produced during two weeks' incubation was lessened in proportion to the size of the application of crude petroleum. In comparing the two sets of samples, it is seen that the soil had in a measure regained its power of nitrate production at the time the last set of samples was taken.

Total counts of aerobic bacteria were much increased by the applications of crude petroleum, the increase being greater with the larger applications of crude petroleum. Although total aerobic counts were slightly higher at the second date of sampling, the percentage of increase, due to the application of crude petroleum, was no larger. Although there was an increase in the total aerobic count of bacteria, the number of bacterial types was very greatly reduced by the higher applications of crude petroleum.

Applications of crude petroleum seemed to have little effect on the number of organisms developing under anaerobic conditions.

Yield records on a plot of such small size are of very little value. However, it was noted that the largest and best ears of corn were produced on the hills which were treated with the highest amounts of crude petroleum. While the number of hills in each treatment is not large enough to give accurate crop data, this does indicate that the crude petroleum as applied in this experiment was not detrimental to plant growth and development.

LABORATORY STUDIES ON AMMONIFICATION AND NITRIFICATION

This experiment was designed to determine the effect of applications of crude petroleum on the biochemical activities of the soil flora as measured by ammonification and nitrification. Also an attempt was made to determine what modifications were brought about in the types of bacteria developing on agar plates.

A brown sandy loam from an old soy bean field in the Purdue Experimental Plots was used in this test.

Nine 100-gm. portions were placed in glass tumblers and 1 per cent of cotton seed meal added. To three of these portions 2 cc. of crude petroleum was added and to three others, 5 cc. was added. The crude petroleum in each case was thoroughly mixed with the soil. The tumblers were then set away to incubate for varying periods of time.

The initial sample and the incubated portions were all analyzed for nitrates, ammonia and bacterial counts.

Bacterial plates were incubated for 10 days and then counted and photographed in order to record the number of colonies and the uniformity of type.

The treatments of crude petroleum used in this experiment were intentionally made very high in an effort to intensify any effects which might be produced.

A study of table 2 shows the ammonia content of the soil to have remained about constant during the first 7 days of incubation, with a slight loss in the case of the treated samples. At the end of 17 days the ammonia content had risen very sharply and this increase continued over the succeeding 10 days of incubation.

Mold growth in all of these samples was very intense and the production of ammonia is probably, in part at least, due to this factor.

However, the untreated sample shows a greater ammonification at all stages than either of the others, indicating that the applications of crude petroleum slightly decreased the ammonifying efficiency of the soil.

Nitrate production was affected to a far greater extent than ammonia production. The formation of nitrates in the samples stopped and the initial nitrates of the soil disappeared.

In contrast to the lowered efficiency of ammonia production and the complete loss of nitrates, bacterial numbers are shown to have increased more rapidly in the treated samples than in the untreated one. A careful study of table 2 shows, however, that the initial effect of the higher application of crude petroleum was to reduce the bacterial count, but that this period of initial decrease was followed by a period of very rapid multiplication.

Of equal interest is an observation of the types of bacteria developing in the treated and untreated samples. Plate 1 shows photographs of petri plate cultures made from various samples at the end of 17 days' incubation. The sample receiving no crude petroleum shows the development of a large number of different types of bacteria, as would be expected in the normal soil flora. Treated samples, however, show in addition to the increased number of colonies, a very marked reduction in the number of bacterial types. With the larger application there is at the end of 17 days' incubation, practically only one type of bacteria developing on the plates. Apparently some property of the crude petroleum has stimulated this particular bacterial type and at the same time has shown an inhibitory action on the other types originally present in the soil.

TABLE 2

Effect of applications of crude petroleum on bacterial counts, ammonification and nitrification

PERIOD OF INCUBATION	AMMONIA ON BASIS OF DRY SOIL			NITRATES ON BASIS OF DRY SOIL			BACTERIA PER GRAM OF DRY SOIL		
	No oil added	2 cc. oil added	5 cc oil added	No oil added	2 cc. oil added	5 cc. oil added	No oil added	2 cc. oil added	5 cc. oil added
days	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*millions*	*millions*	*millions*
0	16.8			4.8			5.7		
7	15.9	15.7	9.1	16.0	3.2	2.2	4.8	10.0	2.6
17	687.0	619.1	450.4	48.0	0.0	0.0	16.5	22.6	34.4
27	775.0	587.3	480.9	80.0	0.0	0.0	28.0	37.8	57.3

Mold growth was very intense in all of these samples as indicated by the presence of white hyphae throughout the soil.

GREENHOUSE EXPERIMENTS

In order that the bacteriological and biochemical activities of the soil as influenced by applications of crude petroleum might be studied in connection with crop growth, a series of experiments was carried out in the greenhouse using 2-gallon pots.

Since previous work had shown the transformation of nitrogen from protein to nitrate to be the chief biochemical factor affected, a rich black loam, high in organic matter was used, to which was added a sufficient quantity of the other fertilizer elements to insure a plentiful supply.

Two series of 15 pots each were set up. The series were duplicates, except for the fact that one received 25 gm. of dried blood per pot in addition to the other substances. Each pot contained 4000 gm. of soil, 20 gm. of calcium carbonate, 10 gm. of potassium chloride, and 15 gm. of calcium acid phosphate.

Crude petroleum was added to each series in varying amounts and was thoroughly mixed with the slightly moist soil before it was placed in the pots. The smallest application of crude petroleum used was 10 cc. per pot and the size of the treatment increased by increments of 10 cc. up to 100 cc. Between 100 cc. and 200 cc. the treatments were increased by increments of 20 cc.

Moisture conditions were kept as nearly optimum as possible but it was found very difficult to get the higher members of the series to take enough water.

At the end of 21 days and again at the end of 60 days, samples were taken from the pots and analyzed for nitrates and bacterial counts.

Decomposition of the dried blood was very intense and during the earlier part of the experiment the surface of the soil was dotted with white mold colonies.

An examination of tables 3 and 4 shows that the nitrate content of the soil in both series was lowered very materially by the application of crude petroleum. In the pots containing the larger applications of crude petroleum, no trace of nitrate could be found. The detrimental effect of the crude petroleum was not so marked in the dried blood series as in the series containing no dried blood. The retardation of nitrification by the crude petroleum treatments had worn off to a large extent at the date of the second and last sampling, i.e., after a period of 60 days.

Bacterial growth was apparently benefited by the applications of crude petroleum. The bacterial count increased with the size of the crude petroleum application in both series.

Uniformity of bacterial type was very marked at the time of the first sampling, particularly in the pots receiving the higher applications of crude petroleum. This degree of uniformity was partially lost in the lower pots of both series at the date of last sampling.

After these samples had been taken, the pots were planted with seed from a disease free ear of yellow dent corn. In addition to the original pots, three others were started. Each of these received the same amount of phosphorous, potassium and lime as did the original pots. In addition, one of these received dried blood and another received the nitrate treatments. No crude petroleum was added to these three pots.

Since it had developed that the nitrate production was very seriously interfered with, alternate pots in both series were treated with a solution of sodium nitrate, once each week, during the period of growth. Each pot received 1/8 gm. of sodium nitrate in a weak solution at each application.

The moisture-content was held at as nearly optimum as possible, and it was found that the higher members of the series took water more readily than they had previously.

Qualitative bacterial samples taken at the time of harvesting, and 115 days after treatment with the crude petroleum, still showed a marked uniformity

TABLE 3

Samples taken from greenhouse pots 21 days after treatment

CRUDE PETROLEUM APPLIED PER POT	POTS CONTAINING DRIED BLOOD			POTS WITHOUT DRIED BLOOD		
	Moisture	Nitrates in dry soil	Bacteria per gram of dry soil	Moisture	Nitrates in dry soil	Bacteria per gram of dry soil
cc.	*per cent*	*p.p.m.*	*millions*	*per cent*	*p.p.m.*	*millions*
10	19.4	121.0	8.61	18.3	3.94	2.46
20	19.4	60.0	18.53	17.2	3.95	2.18
30	18.9	35.5	19.07	18.3	1.95	2.65
40	19.6	28.0	16.89	18.5	1.95	3.38
50	18.4	15.0	15.84	17.4	0.00	3.49
60	18.6	15.1	16.21	19.3	0.00	3.71
70	18.6	10.9	21.07	18.7	0.00	3.98
80	21.3	11.1	16.03	19.5	0.00	4.46
90	18.9	7.98	17.28	17.9	0.00	4.28
100	18.6	7.97	24.85	19.3	0.00	4.57
120	18.4	7.96	25.21	17.8	0.00	6.02
140	19.2	3.94	23.29	15.9	0.00	5.83
160	17.6	0.00	26.58	14.2	0.00	6.64
180	17.0	0.00	24.38	13.5	0.00	7.28
200	16.4	0.00	31.02	12.3	0.00	7.29

TABLE 4

Samples taken from greenhouse pots 56 days after treatment

CRUDE PETROLEUM APPLIED PER POT	POTS CONTAINING DRIED BLOOD			POTS WITHOUT DRIED BLOOD		
	Moisture	Nitrates in dry soil	Bacteria per gram of dry soil	Moisture	Nitrates in dry soil	Bacteria per gram of dry soil
cc.	*per cent*	*p.p.m.*	*millions*	*per cent*	*p.p.m.*	*millions*
0				19.3	21.6	1.87
0	20.1	77.0	12.20			
0				18.3	20.8	1.90
10	18.7	816.0	3.41	18.3	8.8	2.21
20	17.4	400.0	4.36	17.6	8.9	2.35
30	20.1	256.0	4.68	19.4	6.4	2.70
40	19.4	296.0	5.14	18.6	4.1	2.68
50	18.9	336.0	4.81	17.3	4.0	2.91
60	17.8	344.0	5.33	16.5	3.2	3.13
70	17.3	312.0	5.96	17.9	1.6	3.30
80	18.5	264.0	6.17	16.9	0.0	2.86
90	18.7	183.0	6.43	17.6	0.0	2.69
100	18.1	144.0	5.32	17.3	0.0	2.95
120	18.9	96.0	4.59	17.3	0.0	2.74
140	18.8	88.0	6.31	18.6	0.0	3.62
160	16.4	94.0	7.28	17.2	0.0	3.75
180	17.6	92.0	5.69	15.3	0.0	4.21
200	17.1	87.0	6.87	14.6	0.0	5.14

of bacterial type in the pots receiving 100 cc. or over of the crude petroleum and not dried blood. In the dried blood series the pots which received 180 cc. and 200 cc. of crude petroleum showed uniformity of bacterial type. All other pots showed as large a number of bacterial types as would be expected in the normal soil flora.

TABLE 5

Petroleum extracted by ether from soils 115 days after treatment

CRUDE PETROLEUM ADDED PER POT	WITH DRIED BLOOD			WITHOUT DRIED BLOOD		
	Extract from 10 gm. dry soil	Extract minus blank	Petroleum recovered	Extract from 10 gm. dry soil	Extract minus blank	Petroleum recovered
cc.	*mgm.*	*mgm.*	*per cent*	*mgm.*	*mgm.*	*per cent*
0	4.1			3.6		
10	18.2	14.1	67.4	13.7	10.1	48.3
80	101.6	97.5	52.5	103.4	99.8	53.8
200	191.6	187.5	45.6	163.4	159.8	38.9

TABLE 6

Growth (dry weight of tops) of dent corn after 56 days

CRUDE PETROLEUM APPLIED PER POT	POTS CONTAINING DRIED BLOOD		POTS WITHOUT DRIED BBOOD	
	No nitrate	Nitrate added	No nitrate	Nitrate added
cc.	*gm.*	*gm.*	*gm.*	*gm.*
0			27.10	
0				22.40
0	41.40			
10	32.70		6.10	
20		26.10		17.90
30	23.50		1.70	
40		27.50		5.60
50	15.60		1.40	
60		35.00		4.50
70	28.60		7.65	
80		27.40		2.90
90	21.60		1.30	
100		23.60		3.10
120	12.65		4.30	
140		15.50		8.60
160	16.20		0.40	
180		7.45		5.40
200	9.85		0.30	

Qualitative nitrate tests, made with Brucin, gave large quantities of nitrates in all pots except those showing considerable uniformity of bacterial type. It seems that nitrates are not formed in the soil to any considerable extent, as long as the effect of the crude petroleum is strong enough to maintain the uniformity of bacterial type.

The extraction of the soil with ether, as shown in table 5, seems to indicate that approximately 50 per cent of the crude petroleum had either been volatilized or broken down into simple substances by the action of certain of the microörganisms found in the soil. Sufficient data have not been secured, however, to substantiate this point.

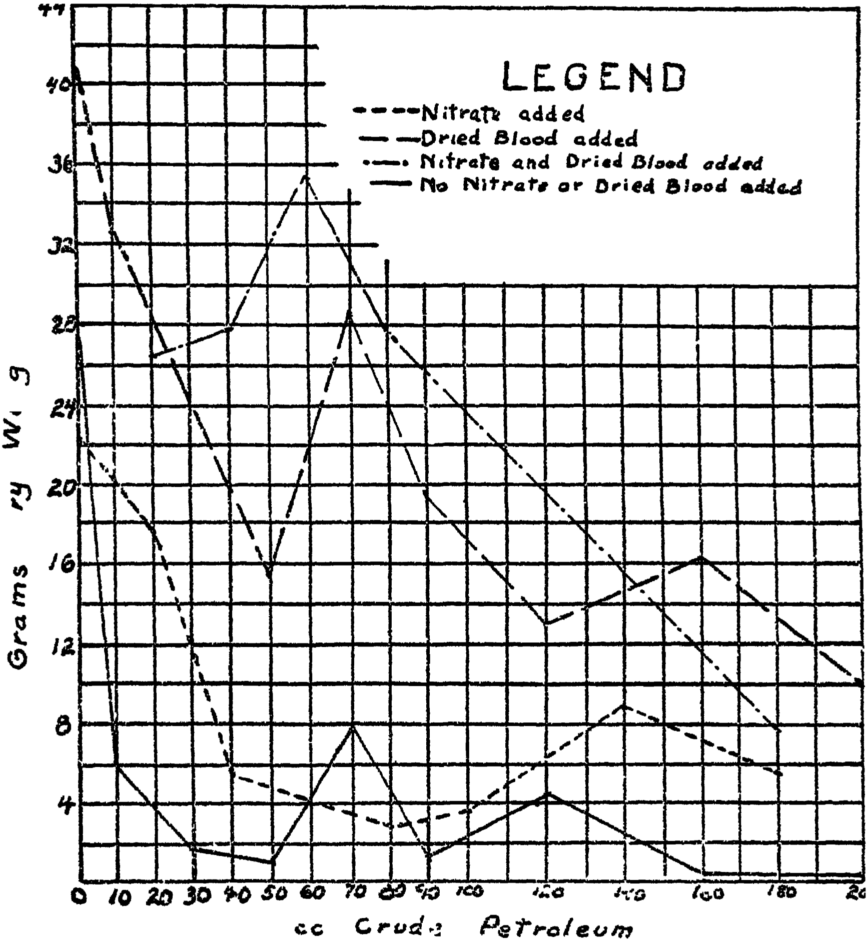


FIG. 1. GROWTH OF DENT CORN IN GREENHOUSE, FIFTY-SIX DAYS OLD

Table 6 and figure 1, illustrating the yield of the dry weight of the corn plants from each pot, show that the addition of an easily soluble nitrate operated to increase plant growth. However, the increase due to the addition of the nitrate was not nearly as large as the increase due to the addition of dried blood to the soil.

The crude petroleum did not seem to decrease the growth of the corn until about 80 cc. to 90 cc. had been applied to each pot.

PURE CULTURE STUDIES

Although several organisms persistently appeared in the petri plates from soil treated with crude petroleum, one type was a great deal more prominent than any of the others. A large number of cultures of this organism were isolated from different soils and studied culturally, morphologically and physiologically.

In this work the outline recommended by the 1920 Report of the Committee on Standard Methods of the Society of American Bacteriologists was followed.

The index number of the organism according to this chart is 5331-52220-1233.

In most respects the culture corresponds very closely to the *Mycobacterium hyalinum* described by Sohngen (5) as a bacterium capable of breaking down and utilizing such compounds as benzene, paraffin and petroleum.

Morphology

Form. The organism is a clear cut rod about three times as long as wide. Long chains of 6 or 8 organisms are occasionally found. Although the growth on agar is slimy and gives indication of capsule formation, none has ever been observed.

Size. The organisms are very uniform in size, being about 1 by 3 microns.

Motility. No motility has been observed.

Spore formation. Spores are not formed.

Staining properties. The organism stains readily but not at all uniformily. Heavily stained granules are usually observed at either end of the rod with Loefflers Methylene Blue. It is Gram negative.

Cultural characteristics

Agar colonies. Colonies on agar develop rather slowly, are medium sized, about 3 to 5 mm. in diameter, elevated, amorphous and glistening.

Agar slant. Growth on agar slant resembles that of the agar colonies, being elevated, glistening and white. It spreads over the surface of the agar and in old cultures tends to form heavy folds or wrinkles of a light yellow hue.

Agar stab. Growth on the surface is in the form of a shiny glistening drop with very slight growth down the line of inoculation.

Gelatin colonies. Colonies resemble the agar colonies very closely. There is no liquefaction of the gelatin.

Gelatin stab. No liquefaction occurs and the growth is rather slow, being slimy and glistening on the surface and very scant along the line of inoculation.

Bouillon. Cloudy growth occurs throughout with the formation of a slight ring around the surface.

Potato streak. Growth is elevated and glistening white, later turning light cream color.

Physiologial characteristics

Arabinose, sucrose, lactose, maltose, raffinose, inulin, and glycerin are not fermented. Dextrose is fermented with the production of slight acidity and no gas. Levulose and mannite give rather high acidity, but no gas formation.

Starch is hydrolysed slowly.

Ammonia is formed from urea, but is not formed from casein, dried blood, gelatin, peptone, egg albumin or cotton seed meal.

Nitrates are reduced to nitrites without the formation of gas.

The organism grows best in the presence of oxygen, but is able to grow in the absence of oxygen to a slight extent.

SUMMARY

1. The soil flora is changed remarkably by applications of crude petroleum. Most types of bacteria are inhibited by the action of the crude petroleum, but some few types are very greatly stimulated by its action. Mold growth is not inhibited by the action of the crude petroleum.
2. Ammonia production in the soil is lowered slightly by applications of crude petroleum. The ammonia produced in the soil is probably the result of mold growth and not bacterial action as the bacterial types favored by the crude petroleum are not able to form ammonia from organic material.
3. When first applied, nitrate production in the soil is completely inhibited by the crude petroleum. The inhibitory action lasts over a varying period of time, depending upon the size of the application, and is followed by a period of rather slow nitrification, which gradually becomes more intense.
4. The data in regard to crop growth are not conclusive, but the indications are that small applications of crude petroleum to the soil do not injure its crop-producing power. Larger applications have a detrimental influence partly because of their effect on the physical condition of the soil.
5. It seems that crude petroleum when incorporated in soil is gradually broken down into simpler products and the effect of its presence is no longer apparent.

REFERENCES

(1) Carr, R. N. 1919 Vegetative growth in soils containing crude petroleum. *In* Soil Sci., v. 8, p. 67.

(2) Gainey, P. L. 1917 Effect of paraffin on the accumulation of ammonia and nitrates in the soil. *In* Jour. Agr. Res., v. 10, p. 355.

(3) Noyes, H. A. 1919 Accurate determination of soil nitrates by the phenol disulfonic acid method. *In* Jour. Indus. and Engin. Chem., v. 11, p. 213.

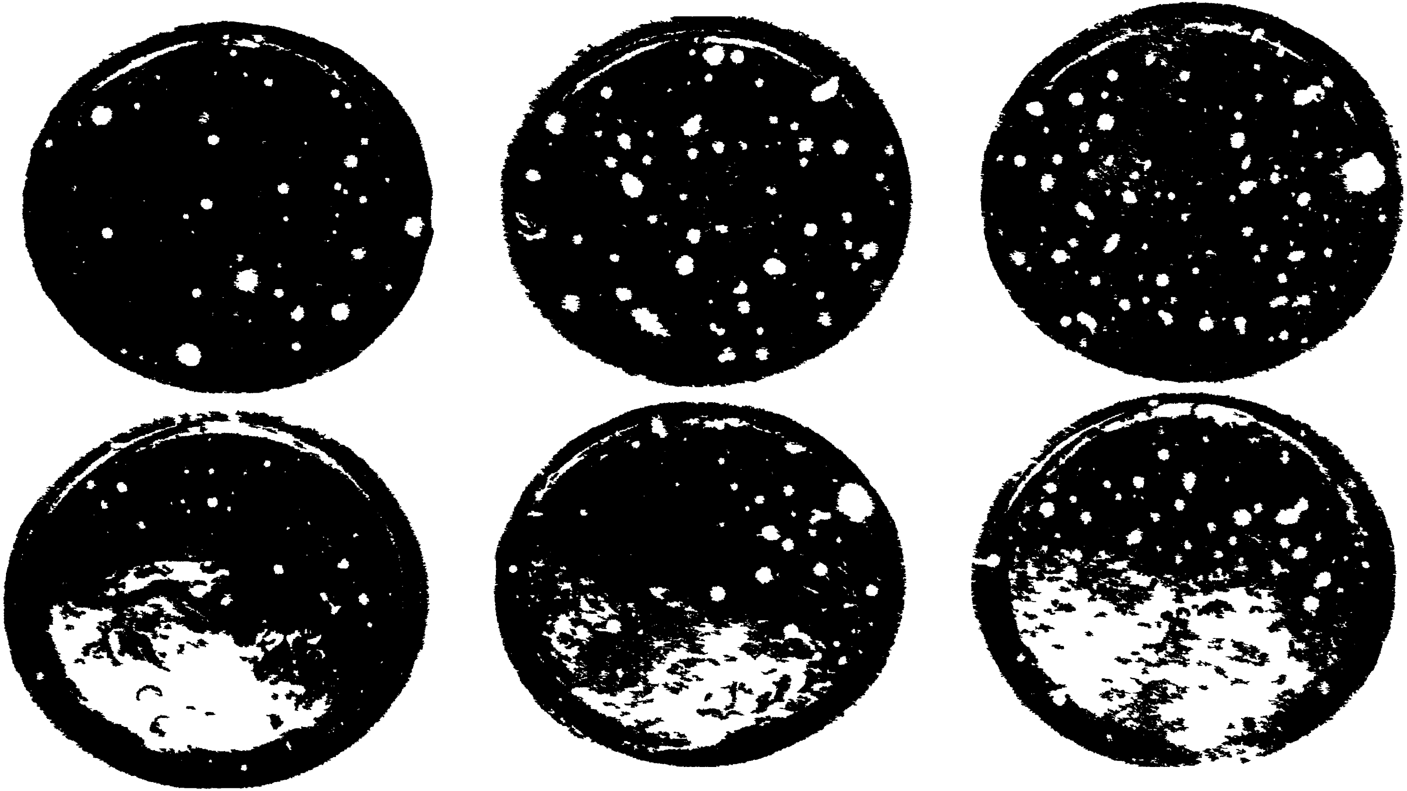
(4) Rahn, O. 1906 Ein paraffin zerstezender Schimmelpilz. *In* Centbl. Bakt. (etc.), Abt. 2, v. 16, p. 382.

(5) Sohngen, N. L. 1913 Benzin, Petroleum, Paraffinöl und Paraffin als Kohlenstoff- und Energiequelle für Mikroben. *In* Centbl. Bakt. (etc.), Abt. 2, v. 37, p. 595.

(6) Tausz, J., and Peter, M. 1919 Neue Methode der Kohlenwasserstoffanalyse mit Hilfe von Bakterien. *In* Centbl. Bakt. (etc.), Abt. 2, v. 49, p. 497.

PLATE 1

Petri Plates Inoculated with 1 cc. of a 1-100,000 Dilution of Soil



ACID PHOSPHATE PRODUCTION BY THE LIPMAN PROCESS: I. EFFECT OF MOISTURE CONTENT OF SULFUR-FLOATS-SOIL MIXTURES ON SULFUR OXIDATION ACTIVITIES[1]

JACOB S. JOFFE

New Jersey Agricultural Experiment Stations

Received for publication September 22, 1922

The Lipman process of mixing rock phosphate, sulfur and some other inert material for the purpose of making acid phosphate is based upon the phenomenon of the oxidation of sulfur by microörganisms, chiefly *Thiobacillus thiooxidans* (6, 10). A number of investigators (1, 2, 3, 4, 13), have followed up the pioneer work of Lipman and his associates (8, 11). Their achievements have been negligible in comparison with the hopes and faith voiced by Lipman in his early work and boldly maintained by McLean (11). All, however, felt the possibilities of making acid phosphate by a bacterial process, eliminating thereby first the sulfuric acid production for acid phosphate production, and secondly the elimination of the acidulation process of the rock phosphate. The idea may sound far fetched but it is the firm belief of the writer that this is possible and the experiments reported below will show how the process may be developed. The chief failure of the investigations including even those of Rudolfs (12) conducted in France almost parallel with the writer's work, is their failure to appreciate some of the fundamental reactions involved in the oxidation of sulfur by microörganisms. It is true that these reactions were overlooked even by Lipman and McLean, but their work was pioneer work. Yet a thorough examination of their work will show how comprehensive in scope it was, how systematically it was attacked, how thoroughly some of the features were looked into, and how many phases of the work were touched. Their followers simply checked up their work and as a result very little progress has been made toward attaining a way of making acid phosphate by the Lipman process.

In the case of the sulfur-floats mixtures, the reactions that take place when sulfur is oxidized to sulfuric acid and when this acid reacts with the tricalcium phosphate are:

(1) $2S + 2H_2O + 3O_2 = 2\ H_2SO_4$

(2) $Ca_3(PO_4)_2 + H_2SO_4 + 2\ H_2O = Ca_2\ H_2\ (PO_4)_2 + Ca\ SO_4 \cdot 2\ H_2O$

(3) $Ca_2\ H_2\ (PO_4)_2 + H_2SO_4 + 2\ H_2O = CaH_4\ (PO_4)_2 + CaSO_4 \cdot 2H_2O$

---

[1] Paper No. 103 of the Journal Series, New Jersey Agricultural Experiment Stations, Department of Soil Chemistry and Bacteriology.

This paper will appear in Rutgers College Studies, vol. 1.

Equation (1), when analyzed from a stoichiometric standpoint, tells us that with the progress of the sulfur oxidation water is taken out from the system for the formation of the sulfuric acid. None of the workers on the problem of securing an ideal mixture for the rapid transformation of the insoluble phosphates have taken this factor in consideration. Their procedure was to weigh the cultures weekly or at other intervals of time, consider the loss in weight as the measure of water lost by evaporation, and add only this much water for maintaining a proper moisture-content. In reality, however, there was an additional loss of culture-moisture equivalent to the weight of the water used for building up the sulfuric acid molecule and the water of crystallization in gypsum plus the weight of the oxygen necessarily introduced for the formation of the sulfuric acid.

$$\begin{aligned} 2S + 3O_2 + 2\,H_2O &= 2\,H_2SO_4 \\ 64 + 96 + 36 &= 196 \end{aligned}$$

Thus from 64 units of sulfur 196 units of sulfuric acid are formed, or for every gram of sulfur, 3.02 gm. of sulfuric acid, a gain in weight of more than 2 gm. for every gram of sulfur oxidized. Also, as indicated, in the second and third equations, one of the end products of the reaction is gypsum, each molecule of which holds two molecules of water of crystallization. This again causes the system to gain weight and minimize the apparent loss by evaporation. The cultures, therefore, suffered a constantly progressing deficiency in moisture and oxidation was necessarily retarded. Experiments conducted by the writer with a view of studying the gain in weight in cultures of progressive sulfur oxidation proved this theoretical consideration beyond doubt. In sand cultures a gain very close to the theoretical was obtained while with some mixtures of rock phosphate, sulfur and soil the gain at times was below the theoretical probably due to the liberation of CO_2 from some of the carbonates present in the mixtures. Thus the moisture-content of the mixtures is a function of the sulfur oxidized.

Another point which the first equation brings up is the oxygen supply in the process of sulfur oxidation. This point has been appreciated by Lipman and McLean; but Rudolfs (12) contrary to all expectations, from a theoretical point of view, found that aeration had no stimulating effect on the oxidation of sulfur. It might be suspected that some other factor, perhaps the one of the proper moisture-content, was the cause of the failure. Experiments on the effect of aeration have already been reported elsewhere (6).

The reactions involved in the conversion of rock phosphate into soluble forms by means of acid as represented empirically in equations (2) and (3), belong to the heterogeneous system and have been studied very little. Throughout the work it was noticed that the cultures reached a hydrogen-ion concentration as expressed by the Sörensen figure pH 2.8. A test of the chemically pure primary calcium phosphate as well as that of the commercial acid phosphate also gave a pH 2.8. Naturally the speeding up of the initial stage of incubation in order to reach such a pH was considered of importance. Experiments were conducted to see whether additions of sulfuric acid at the start will bring about such a condition. These experiments have been reported already (7).

An important step towards the successful accomplishment of making acid phosphate by the Lipman process was the building up of mixtures of rock phosphate, sulfur and some other inert material, whereby the total and the available phosphate content should both be high.

EXPERIMENT 1. EFFECT OF MOISTURE CONTENT

A mixture in the proportions of 15 gm. of rock phosphate flour, 5 gm. of sulfur and 80 gm. of greenhouse soil was distributed into small earthenware pots in 400-gm. portions. Pots 1 and 2 received water equal to 50 per cent of the total moisture-holding capacity; 3 and 4, 60 per cent; 5 and 6, 75 per cent; 7 and 8, 50 per cent. With the exception of pots 7 and 8 the moisture-content was kept up, by weekly determinations to the amount added at the start. Pots 7 and 8 were weighed carefully, and the loss in weight was considered as the loss by evaporation. Results are given in table 1.

The results presented are very striking and bring out several interesting points. First, McLean's (11) results that 50 to 60 per cent saturation of the mixture is the best are confirmed. Pots 1 and 2 show this very clearly. Second, the failure of some investigators to get results even with 50 per cent saturation is due to the erroneous calculation of the moisture lost by evaporation, as pointed out above. After the third week the importance of the moisture factor begins to show, although the oxidation of sulfur is still going on, as indicated by the gradual accumulation of soluble phosphorus. The limiting factor at such a moisture-content is not of a direct nature. In another part of this work to be reported later the physico-chemical principles of the course of conversion of insoluble phosphates into soluble forms are expounded and it is pointed out that in such a system the speed of diffusion of the reacting substances and the amount of contact of the reacting substances play an important rôle. In cultures 7 and 8 there may have been sufficient moisture for the metabolic processes of the organisms responsible for the oxidation of the sulfur, but the diffusing power and amount of contact is lowered. Third, cultures 3 and 4 lagged behind until the pH went down to 2.8, but gained rapidly after reaching this point. It has been pointed out elsewhere (6) that up to the pH 2.8, the sulfur-oxidizing organisms have to compete with other groups of microörganisms. It is very likely true that at a 50 per cent saturation the sulfur-oxidizing flora is at an ad-

TABLE 1

Effect of moisture content on availability of rock phosphate in composting it with sulfur and soil

POT NUMBER	AFTER 1 WEEK		AFTER 2 WEEKS		AFTER 3 WEEKS		AFTER 4 WEEKS		AFTER 5 WEEKS		AFTER 6 WEEKS		AFTER 7 WEEKS		AFTER 8 WEEKS	
	Reaction	Soluble P in 100 gm. of mixture	Reaction	Soluble P in 100 gm. of mixture	Reaction	Soluble P in 100 gm. of mixture	Reaction	Soluble P in 100 gm. of mixture	Reaction	Soluble P in 100 gm. of mixture	Reaction	Soluble P in 100 gm. of mixture	Reaction	Soluble P in 100 gm. of mixture	Reaction	Soluble P in 100 gm, of mixture
	pH	*mgm.*	*pH*	*mgm.*	*pH*	*mgm.*	*pH*	*mgm.*	*pH*	*mgm.*	*pH*	*mgm.*	*pH*	*mgm.*	*pH*	*mgm.*
1	5.0	32.1*	3.4	43.1	3.2	69.2	2.8	92.8	2.8	104.9	2.6	128.7	2.6	149.7	2.4	171.3
2	5.0	31.3	3.4	42.7	3.0	69.9	2.8	94.7	2.6	111.7	2.6	134.6	2.6	150.4	2.4	170.1
3	5.2	30.4	3.8	34.0	3.6	58.7	3.0	89.2	2.8	112.3	2.6	142.7	2.4	169.3	2.2	189.4
4	5.2	29.6	3.8	36.2	3.4	57.9	3.0	90.3	2.8	116.4	2.6	139.6	2.4	168.1	2.2	186.2
5	5.6	28.7	4.2	30.6	4.0	42.1	3.8	43.7	3.8	41.9	3.4	54.2	3.0	59.7	2.8	69.3
6	5.6	27.9	4.4	31.0	4.0	41.6	3.8	44.3	3.8	42.7	3.0	59.6	3.0	61.3	2.8	70.7
7	5.0	32.1	3.4	43.0	3.0	63.6	2.8	84.2	2.8	91.6	2.8	96.7	2.8	103.4	2.6	119.1
8	5.0	32.2	3.2	44.0	3.0	61.7	2.8	83.9	2.8	87.2	2.8	94.9	2.8	101.3	2.8	111.7

* Total phosphorus content of mixture at start was 203 mgm. per 100 gm. of mixture.

vantage over others, while after reaching the pH 2.8 the field of action is clear and then a higher moisture-content is just what is required for proper diffusion. Fourth, a more nearly saturated condition, as the 75 per cent saturation was in this case, is detrimental to the sulfur oxidizing flora, especially in the early period of incubation.

From this experiment, therefore, it appears that for ideal sulfur oxidizing conditions the cultures should be started with a moisture-content of 50 per cent saturation, and after the reaction reaches a reaction of pH 2.8 the moisture-content should be gradually raised to a 60 per cent saturation.

REFERENCES

(1) Ames, J. W., and Richmond, T. E. 1918 The effect of sulfofication and nitrification on rock phosphate. *In* Soil Sci., v. 6, p. 351–364.

(2) Brown, P. E., and Gwinn, A. P. 1917 Effect of sulfur and manure on the availability of rock phosphate. Iowa Agr. Exp. Sta. Res. Bul. 43.

(3) Brown, P. E., and Warner, H. W. 1917 The production of available phosphorus from rock phosphate by composting with sulfur and manure. *In* Soil Sci., v. 4, p. 269–282.

(4) Ellet, W. B., and Harris, W. G. 1920 Coöperative experiments for the composting of phosphate rock and sulfur. *In* Soil Sci., v. 10, p. 315–325.

(5) Joffe, J. S. 1922 Preliminary studies on the isolation of sulfur-oxidizing bacteria, etc. *In* Soil Sci., v. 13, p. 161–172.

(6) Joffe, J. S. 1922 Studies of sulfur oxidation in sulfur-floats-soil mixtures. *In* Soil Sci., v. 13, p. 107–118.

(7) Lipman, J. G., and Joffe, J. S. 1920 The influence of initial reaction on the oxidation of sulfur and the formation of available phosphates. *In* Soil Sci., v. 10, p. 327–332.

(8) Lipman, J. G., McLean, H. C., and Lint, H. C. 1916 The oxidation of sulfur in soils as a means of increasing the availability of mineral phosphates. *In* Soil Sci., v. 1, p. 533, 539; v. 2, p. 499–538.

(9) Lipman, J. G., and McLean, H. C. 1918 Experiments with sulfur phosphate composts, conducted under field conditions. *In* Soil Sci., v. 5, p. 243–250.

(10) Lipman, J. G., Waksman, S. A., and Joffe, J. S. 1921 The oxidation of sulfur by soil microörganisms. *In* Soil Sci., v. 12, p. 475–489.

(11) McLean, H. C. 1918 The oxidation of sulfur by microörganisms in its relation to the availability of phosphates. *In* Soil Sci., v. 5, p. 251–290.

(12) Rudolfs, W. 1921 Experimental studies on sulfur oxidation by microörganisms. Thesis, Rutgers College, New Brunswick, N. J.

(13) Shedd, O. M. 1919 Effect of oxidation of sulfur in soils on the solubility of rock phosphate and on nitrification. *In* Jour. Agr. Res., v. 18, p. 329–345.

A NEW METHOD OF MECHANICAL ANALYSIS OF SOILS

D. S. JENNINGS, M.D. THOMAS, AND WILLARD GARDNER

Utah Agricultural Experiment Station

Received for publication July 10, 1922

From the beginning of scientific work on soils it has been recognized that a mechanical analysis is a necessary part of any complete soil description. There is a tendency for investigators in soils to seek for generalized characteristics, such, for example, as the effective radius, the moisture equivalent, and the hygroscopic coefficient. It is evident, however, that the ultimate solution of many of the problems in soil physics and chemistry must depend upon information which will come with a greater knowledge of the mechanical states and processes of the soil in their microscopic detail. Although many serious efforts have been made to perfect a suitable method of mechanical analysis, it can scarcely be said that satisfactory results have been obtained.

The elutriation and sedimentation methods which have been described are so laborious and time-consuming that their use for obtaining the complete frequency distribution curve is quite out of the question except in certain special cases. The standard methods of this type have been studied and compared by Joseph and Martin (4) who recommend the use of sodium carbonate as the deflocculating agent and gravity sedimentation as the most convenient means of effecting the separation. The various methods of obtaining the distribution curve have been summarized by Wightman and Sheppard (12) who also submit some interesting results with silver halide suspensions obtained by two optical methods. Since developing the method described in this paper, we have had access to the recent papers of Odén (6, 7, 8). He has elaborated the fundamental idea of the simple sedimentation method of Schloessing (11) and devised an ingenious procedure for obtaining the complete distribution curve of soils. He suspends a balance pan near the bottom of a cylinder containing the soil suspension and measures the rate of settling of the material on the pan. From the "accumulation" curve so obtained the distribution curve is calculated. The method we propose has been developed primarily as a result of the need, long felt at this Station, for a simple means of determining the amount of very fine material in soils, particularly the particles smaller than one micron in diameter. This method is also applicable to the fine sand and silt. The authors feel that it offers many advantages over other methods.

APPARATUS AND TECHNIQUE

The method is simple. It consists in shaking a dilute, fully deflocculated soil suspension in a cylindrical vessel, placing the latter in an upright position, and determining the concentration as it changes with the time at measured distances below the surface. We have found an ordinary Oldberg percolator, with a centimeter scale on the outside, convenient for this purpose. The sample to be analyzed is drawn off from below through a permanently fixed multiple-intake pipette with the openings turned in a horizontal direction, as illustrated in figure 1. The capacity of this pipette is made small in comparison with the size of sample to be drawn off and the rate of flow is controlled by a glass nozzle at the outlet. We make a practice of discarding the first small fraction standing in the pipette together with a small additional amount used for washing. The percolator we have used is 40 cm. high by 8.5 cm. in diameter, and the pipette with nine intakes has a capacity of about 2 cc. As indicated below, the limit of precision is in the sampling and weighing, and it is therefore necessary to use every precaution for accuracy in these operations. It is possible that the nephelometer may be used with greater speed and precision for determining the suspension density when only very small particles are present, and we hope to investigate its use for this purpose.

We have found that when the rate of flow of the sample is about 15 cc. per minute, the currents set up in the cylinder are not strong enough to cause appreciable mixing, and it is possible to go on sampling at frequent intervals without shaking the suspension after each sampling. This procedure has been successfully adopted when all the particles with a radius larger than 5μ have had time to fall below the intake of the pipette. The larger particles, however, fall so rapidly that the sample must be taken more quickly when they are present in order that the ratio of the time of sampling to the total time of settling may be small and in order that the stream lines may be strong enough to carry the large particles into the sampler. We therefore use as high a column of liquid as possible at first, omit the nozzle, and shake the suspension after each sampling. The position of the surface is read before and after each portion is drawn off.

The problem of attaining and maintaining a completely deflocculated condition in the suspension is vital to this method. Odén (7, p. 332) recommends a concentration of 0.007 to 0.02 per cent of ammonia in the liquid after the usual preliminary shaking. Arid soils generally contain so much calcium carbonate and soluble salts that his method fails in its purpose. We have therefore adopted with promising success, the recommendation of Joseph and Martin (4), of using sodium carbonate as the deflocculating agent. This salt diminishes the concentration of the calcium ions and at the same time supplies hydroxyl ions to charge the soil particles. It would seem from our limited experience that the best concentration to use is about 0.015 to 0.03 per cent. With smaller and larger amounts flocculation has occurred. We are investigating this point, and another paper is in process on this question.

It is also important in this method to adjust the suspension density within certain limits. On the one hand the concentration must not be so high that the particles interfere with each other appreciably in their fall, and on the other hand it must not be so low that a suitable sample does not contain enough solid material to weigh accurately. Odén (8) states that 1 per cent is not too high a concentration. Our experimental data indicate that about 0.2 per cent of material below 5μ radius is appropriate, but this point needs further investigation.

Since the rate of fall of a particle is inversely proportional to the viscosity of the liquid, and since, as Robinson (10) points out, the latter is increased about 30 per cent by a decrease in temperature of 10°, it is obvious that a large error may be introduced through a failure to control the temperature. This error may be further increased by mixing due to convection currents caused by unequal heating of the system. Instead of using a thermostat we have set up our apparatus in a deep basement room in which the temperature has been found to vary but slightly. The tubes have also been properly insulated in order to prevent the influence of local temperature changes.

DISCUSSION OF THEORY

Physical basis

This method involves the assumption that the particles fall as individuals at a constant rate irrespective of the presence of other particles. It classifies the soil into fractions according to their rates of fall through the liquid and, in order to approximate the size of these particles we make use of Stokes' equation

$$v = Cr^2 \qquad (1)$$

where

$$C = \frac{(2/9)\, g\, (\rho - \rho')}{\eta}$$

In this equation v is the rate of fall of spherical particles of radius r and density ρ in a liquid of viscosity η and density ρ'. Knott (5) has pointed out that the use of Stokes' equation in this sense serves only to define the effective radius.

If any irregular particle is thought of as being molded into a sphere, the radius of the sphere may be regarded as the radius of the particle. This will differ slightly of course from the value determined by the above equation, not only because the particle is not spherical in shape but also because its density may differ from the mean value for the entire sample. These difficulties are inherent in any sedimentation method. We shall designate the value of r that satisfies equation (1) as the "equivalent" radius. Odén (6) assumes that the density of the sample is constant in his definition of "effective" radius. It should be noted that the term "effective" radius is used in soils literature in another sense, and the term "equivalent" radius is probably preferable in this connection.

It is well known that the density of the mineral material of a soil is practically independent of the size of the particles so that the variation in density throughout the sample will be small. The organic matter offers another problem that deserves special study. Odén (6), Hall (3), and other investigators (2, 12) have concluded that the rate of fall of an irregularly shaped particle is not materially different from that of a sphere of the same volume and density.

The upper limit of application of Stokes' law is given by Allen (1):

$$r^2 = \frac{9\ \eta^2}{2\ g\rho'\ (\rho - \rho')}$$

and represent a radius of about 85μ in the case of ordinary soil particles. The lower limit has not been determined, but Perrin (9) has shown that the law holds for particles to about 0.2μ radius.

The calculation

The suspension density at any point *ah* (fig. 1) beneath the surface is due to the presence of a large number of different sized particles, and the decrease in this aggregate concentration is due to the disappearance one by one of these separate species. The concentration of the particles of any given size at this point will remain constant until those particles (of this size) which were originally at the surface have had time to fall through the distance *ah*. The concentration of this species will then decrease abruptly to zero. In other words each species of particles may be thought of as falling as a solid column, independently of all the others.

The difference between two successive measurements of the suspension density q' will represent the amount of a fraction whose limiting dimensions are given by the radii corresponding to the two times at which the measurements are taken. If therefore the relation between q' and t is obtained experimentally, we may calculate the equivalent radius by Stokes' law:

$$r = \sqrt{\frac{ah}{Ct}} \qquad (1')$$

and read at once from the graph representing the $(q' - r)$ relationship the amount of the sample lying between any two values of r. If we wish to express the suspension density as a fraction part of the original concentration q_o, we may plot the ratio $\left(\frac{q'}{b}\right) = q$), against the radius r, and read the percentage of any fraction from the graph.

If the weight (w') of particles of radius r in the sample is represented graphically as a continuous function of r (see figure 2) we may write:

$$dq' = \left(\frac{w'}{L}\right) dr \qquad (2)$$

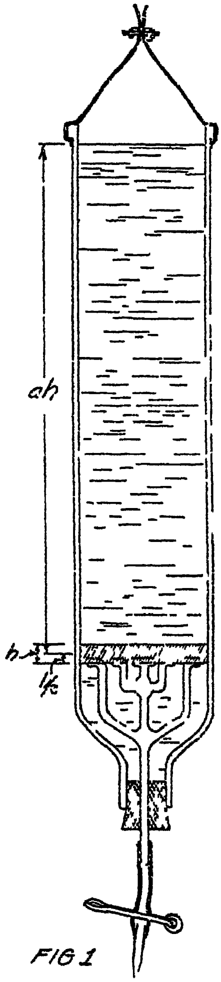


FIG 1

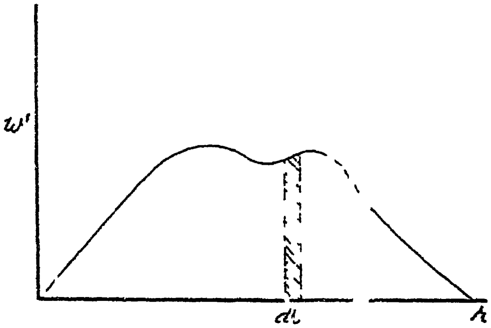


FIG 2

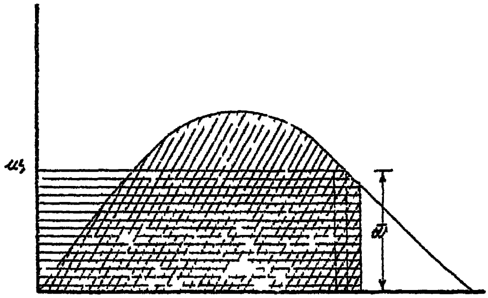


FIG 3

FIG. 1. SECTION OF APPARATUS

FIG. 2 AND FIG. 3. DISTRIBUTION CURVES ILLUSTRATING METHOD OF CALCULATION

where L is the volume of the liquid in which the soil is suspended. Or, it W is the total weight of the sample, we may write for convenience:

$$\left(\frac{w'}{W}\right) = w, \left(\frac{q'}{q_o}\right) = q, \text{ and } \left(\frac{W}{L}\right) = q_o$$

equation (2) will then become

$$\frac{dq}{dr} = w \tag{3}$$

Using this equation we may calculate the $w-r$ curve from the slope of the $q-r$ curve.

Precision

In figure 4 is shown a group of hypothetical curves (series 2) for which the equations are known, representing in their general form possible $w-r$ distribution curves, and in series 1 the $q-r$ curves from which they were obtained by the above transformation. From the form of equation (3), it is obvious that the measure of precision in the determination of the $w-r$ curves is the same as that of the slopes of the original series 1 and the magnitude of the probable error may be estimated from the following. If n cc. are drawn off in the sample to be used for the determination of q', the total weight m of soil remaining after the water has been evaporated will be given by the equation:

$$m = \frac{nq'}{L}$$

If e represents the probable error of the weighing of the sample, the fractional error E will be:

$$E = \frac{}{nq'/L}$$

In the determination of the slope of the curves the difference in ordinates must be measured corresponding to different values of r, but inasmuch as the error in the latter may be controlled almost entirely by the choice of the scale on which the data are plotted, the error will arise principally from the error in the determination of q'. At the highest point on the curves of series 1 of figure 4, the width of the line is of the order of $\frac{1}{250}$ of the ordinate, so that an experimental error of this magnitude should not fall appreciably off the curve.

In order to sample the suspension it will be necessary to draw off a finite stratum of thickness h (see figure 1), and if h is small the suspension density may be said to vary linearly over this range and the sample to represent the concentration at the middle point of the section. A small error will be introduced by this assumption since the density is not strictly a linear function of the depth over this range. The magnitude of the error may be estimated as follows:

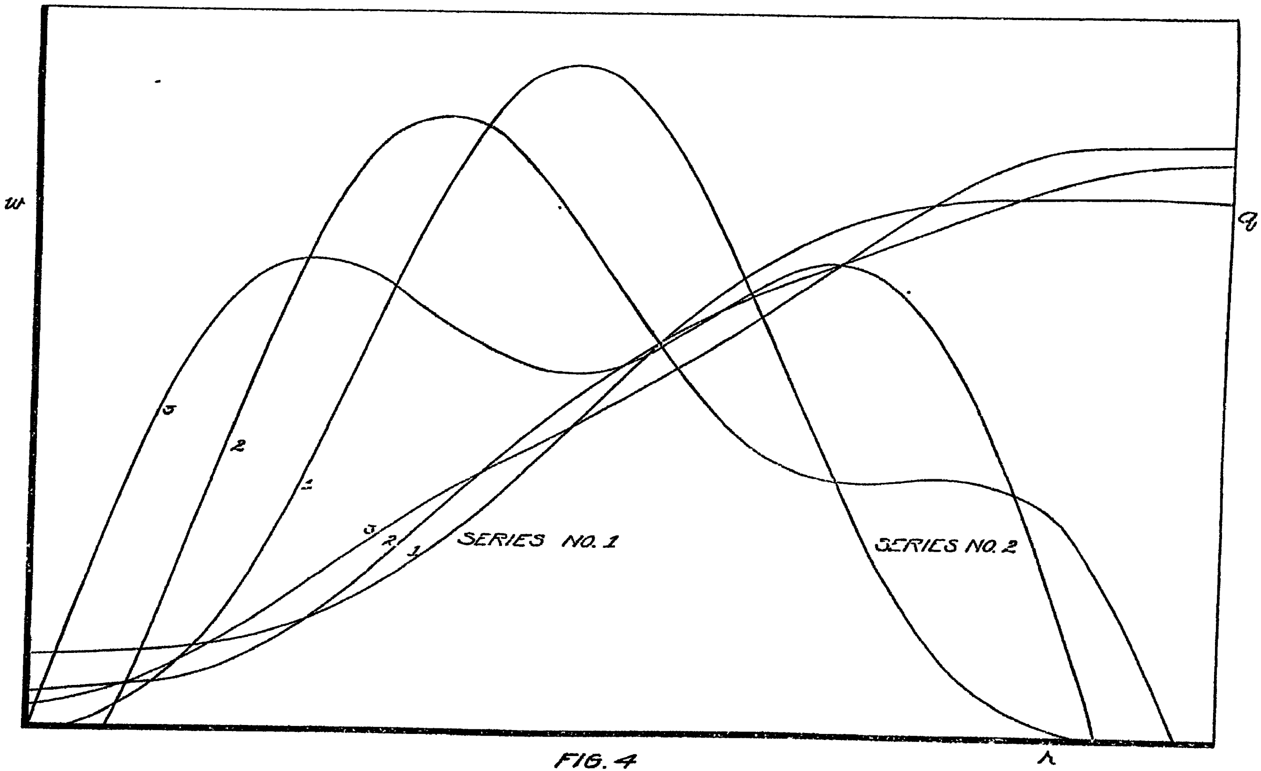


Fig. 4. Illustrative Distribution Curves (Series 2) and the Hypothetical Curves (Series 1) from Which They Were Obtained

These curves serve to indicate the way in which detail in the distribution curves may be suppressed in the experimental curves from which they are calculated.

If r_1 = radius of particle of species vanishing at a distance ah beneath the surface at time t

q_1 = density of suspension at this point at time t

r_2 = radius of particle of species vanishing at point $ah + \frac{bh}{2}$ at time t where b is a fraction

q_2 = density of suspension at point $ah + \frac{bh}{2}$ at time t

$\bar{w}$ = mean value of w for interval $r_2 - r_1$

We may then write

$$\frac{q_2 - q_1}{q_1} = \frac{\bar{w}\,(r_2 - r_1)\,(r_1)}{\int_o^{r_1} wdr\,(r_1)} \tag{4}$$

By means of equation (1′) we may eliminate the ratio

$$\frac{r_2 - r_1}{r_1}$$

by substituting in its place the ratio

$$\frac{\sqrt{a + b/2} - \sqrt{a}}{\sqrt{a}}$$

We may also substitute R for the ratio

$$\frac{\bar{w}r_1}{\int_o^{r_1} wdr}$$

and equation (4) will become

$$\frac{q_2 - q_1}{q_1} = \frac{R\,(\sqrt{a + b/2} - \sqrt{a})}{\sqrt{a}} \tag{4′}$$

From figure 3 it may be seen that the ratio R is equal to the ratio of the rectangular portion to the area of the curve from 0 to the point r_1. This magnitude will therefore vary through a small finite range not far from unity, whereas the other factor of the right hand member of (4)′ may be made as small as desired consistent with other considerations governing the choice of the factor a. The small particles will of couse move slowly, and the depth from which the sample is taken must therefore be governed by this consideration. For a value of a = 10 cm., this ratio is of the order 0.025 if we give to b the value unity. As a matter of fact b must be less than unity and in the ordinary case will be a small fraction. For $b = 0.1$ this ratio will become of the order 0.0025.

Another source of error which may be significant for large values of r arises from the fact that in securing the sample for analysis the heavier particles may not all follow the stream lines into the pipette, and a certain fractional part of each species may fall below the intake. If we break up the quantity q' into its component parts, q_1', q_2', q_3', etc., and if we lose an amount α_1 of species 1, α_2 of species 2, α_3 of species 3, and so on, we shall have for two successive *measured* values of q':

$$q'_a = \Sigma\{(q_1 - \alpha_1) + (q_2' - \alpha_2) + \ldots.\}$$

$$q'_b = \Sigma\{(q_2' - \alpha_2) + (q_3' - \alpha_3) + \ldots.\}$$

In measuring the slope of the curves the difference of these two values of q' will be the value obtained, whereas the correct values will by $q_1' - q_2' = q_1''$. The ratio of the measured and true values of this difference will therefore be given as

$$\frac{q'a - q'b}{q_1' - q_2'} = \frac{q_1'' - \alpha_1}{q_1''} = 1 - \frac{\alpha_1}{q_1''}$$

so that the fractional error is given by the ration, $\frac{\alpha_1}{q_1}$

EXPERIMENTAL RESULTS

To illustrate the method we are reporting the results obtained for two different types of soil, Greenville silty clay loam and Trenton clay. The former is a good agricultural soil containing a large percentage of fine sand and soil and 1.6 per cent organic carbon, while the latter is a very tight clay containing only 0.65 per cent organic carbon. Both soils are similar chemically and contain about 35 per cent of calcium and magnesium carbonates. The water-soluble material in the two soils is given in table 1.

TABLE 1

Analysis of water-soluble material

SOIL	TOTAL SALTS	CO_3	HCO_3	Cl	SO_4	Ca	Mg
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Greenville silty clay loam.......	0.038	None	0.017	0.003	0.004	0.013	0.003
Trenton clay..................	0.126	None	0.057	0.019	0.038	0.008	0.003

The experiment was carried out as described above, the observations being made at time-intervals of from 40 seconds to two weeks, the depth of the sampling varying from 30 cm. to less than 1 cm. The time of each sampling was then corrected to correspond to a standard depth of 10 cm. Ten grams of soil were shaken for 2 hours with 50 cc. of 0.5 per cent sodium carbonate.[1] The Greenville soil was then diluted to 2 liters and the Trenton to 5 liters. Additional sodium carbonate was added to make up the concentrations noted in the legend of the graph of figure 5. Two entirely independent sets of observations are given with each soil. These agree closely in the case of the lighter soil, but the

---

[1] Subsequent work has shown that two hours' shaking is insufficient to deflocculate certain soils and that oven-dried soils deflocculate more slowly than air-dried soils.

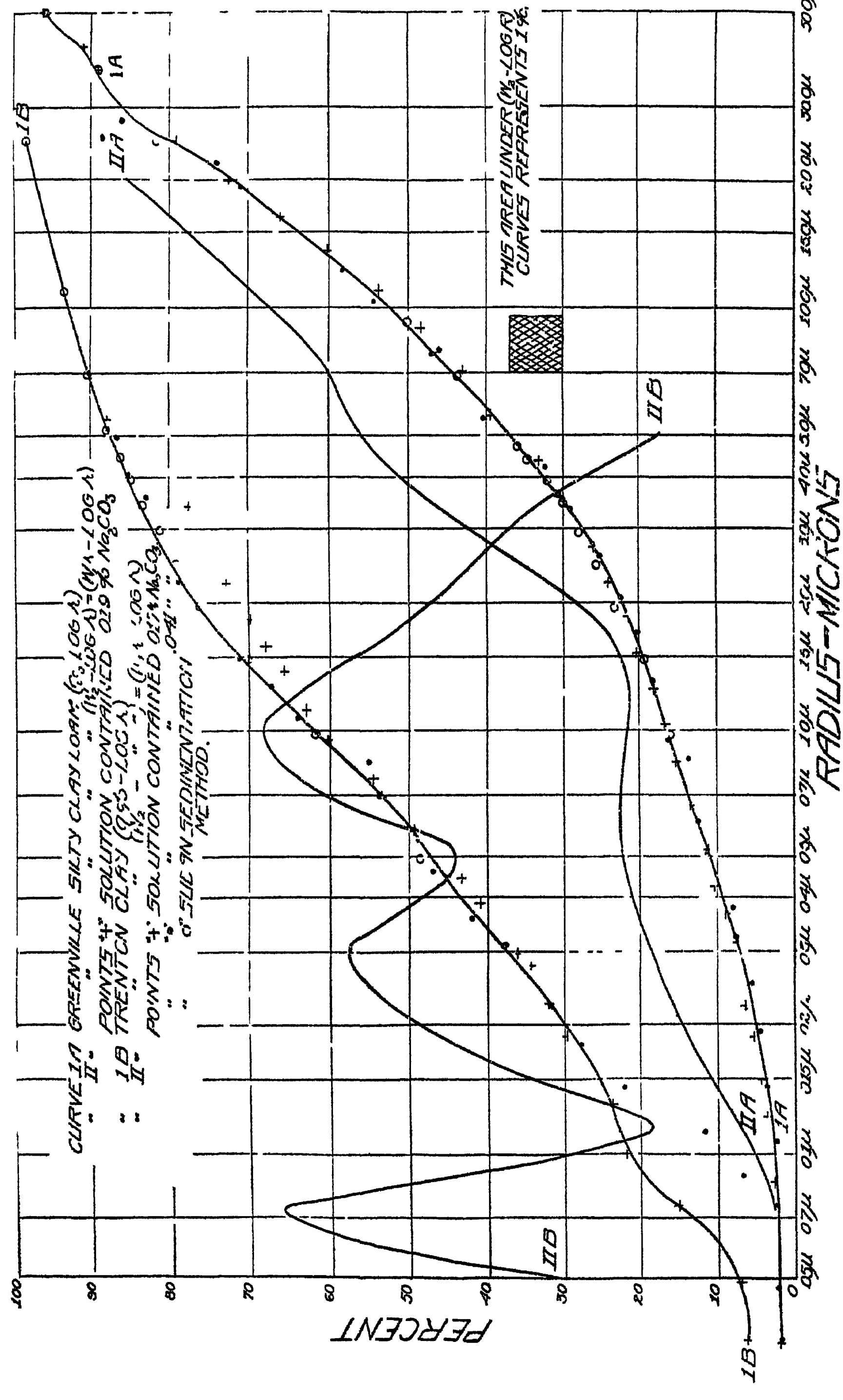


FIG. 5. EXPERIMENTAL DATA AND DISTRIBUTION CURVES FOR TWO SOILS

Trenton clay shows some interesting discrepancies. The system with 0.017 per cent sodium carbonate gives low results in the silt, indicating apparently a partial flocculation of this material. The system with 0.041 per cent sodium carbonate behaves normally for a long time, but there eventually appears to be flocculation of the very fine clay, due perhaps to the high concentration of the sodium ion. It should be noted in this connection that another experiment, not recorded here, in which 0.05 per cent sodium carbonate was used showed flocculation of the clay at an earlier stage of the process. This work indicates the need for careful flocculation studies, particularly in dealing with soils in which soluble salts are present.

As a control on this method, we have made a number of sedimentation separations of these soils by a modification of the method of Joseph and Martin (4), using an 8-ounce bottle to hold the sample and a siphon with the intake turned upward to make the pouring. Individual 2-gram samples were used for each separation, and the sediment remaining when the decantation was complete was dried and weighed. Duplicate determinations almost invariably agreed so perfectly that we consider the method thoroughly reliable. The results thus obtained have been superimposed on the curves obtained by the new method, and it will be observed that the concordance is very good over the range compared, but that the new method gives information concerning a wide range of particles which are too small to be studied by the sedimentation method.

GRAPHICAL REPRESENTATION

The two series of curves of figure 4 illustrate graphically the relation between a function and its derivative and apply to any choice of independent variable. In plotting our experimental data we have used the ratio $\frac{q'}{q_0} = (q)$ as ordinate and the logarithm of the time as abscissa. This is done entirely as a matter of convenience so that all the data may be shown on the diagram (8). Since the logarithm of the time is a linear function of the logarithm of the radius, plotting the one is equivalent to plotting the other and avoids any numerical calculation. Only the values of the radius however, are indicated on the graph (see figure 5).

We have also shown the derivative of this $(q - \log r)$ curve on the same diagram. If therefore we generalize the meaning of equation (3), regarding w as the weight of soil corresponding to any function of r, $[f(r)]$, this equation may be rewritten in the form:

$$dq = w_1 df_1 = w_2 df_2 = w_3 df_3 \quad . \; . \; . \; . \qquad (3')$$

the subscript being used to indicate the choice of independent variable. If therefore $f_1(r) = r, f_2(r) = \log r$, and $f_3(r) = \frac{1}{r}$, we may readily obtain the relation

$$w_2 = w_1 r;\; w_3 = w_1 r^2$$

$$w_2\, d\,(\log r) = w_1\, r\,(dr/r) = w_1\, dr$$

$$w_3\, d\,(1/r) = -\, w_1\, r^2 \frac{dr}{r^2} = -\, w_1\, dr$$

The physical meaning of the logarithmic graph may be somewhat obscure. Another way of condensing the data for large values of r, is to plot its reciprocal as the independent variable. Although this is less convenient than the other method it has an important physical meaning since the reciprocal of the radius is a measure of the specific surface of the particle.

It should be noted that both the $(w_1 - r)$ and $(w_3 - \frac{1}{r})$ relationships can be obtained from the $(q - \log r)$ curve by a simple calculation. It we let

$$y = q$$

and

$$x = \log_{10} r = \log_e r \cdot \log_{10} e$$

$$\frac{dy}{dx} = \frac{dq}{d \log_{10} r} = \frac{r}{\log_{10} e} \cdot \frac{dq}{dr}$$

If

$$R = 1/r$$

$$dR = -\frac{dr}{r^2}$$

and

$$\frac{dy}{dx} = \frac{1}{r \log_{10} e} \frac{dq}{dR}$$

These curves are worked out in figure 6 from the diagrams of figure 5.

ROUTINE MECHANICAL ANALYSES

As a routine method of making mechanical analyses, the procedure here proposed has many advantages over the standard methods. The units of apparatus are cheap and as many as desired can readily be set up. Indeed only standard equipment such as may be found in any soils laboratory need be used. If the soil is put through a half-millimeter sieve after shaking it with the deflocculating agent or if a separate sample is treated in this way it will be possible to obtain all the conventional groups of sizes below this point except perhaps the separation at 0.25 mm. diameter by merely drawing off a few samples of the liquid at certain times. Table 2 gives the rate of settling through a 10 cm. column at 16°C. assuming the density of the soil to be 2.7.

By increasing the height of the column of liquid at first and decreasing it later a convenient and accurate system of timing the samples could readily

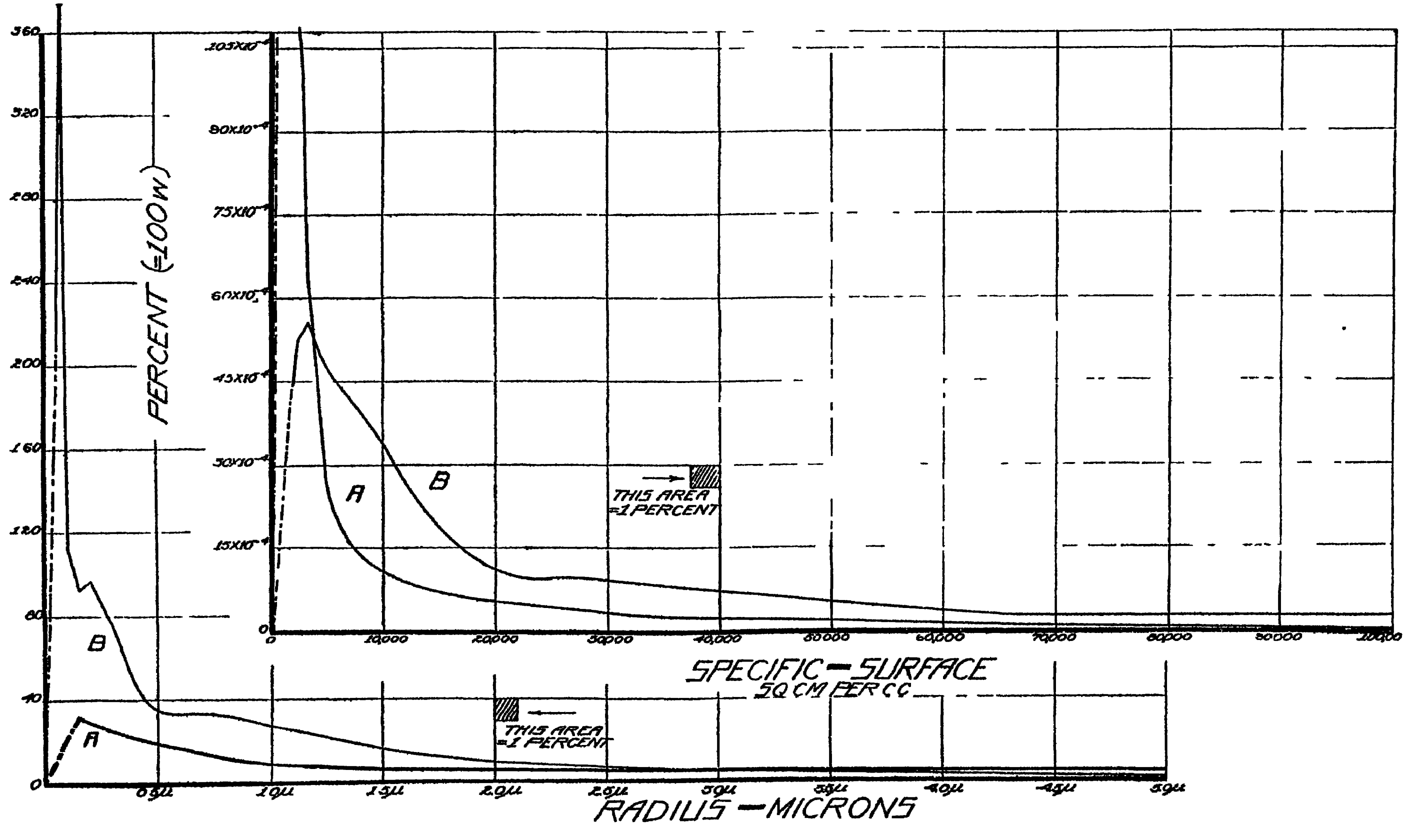


FIG. 6. DISTRIBUTION CURVES FOR TWO SOILS

A, Greenville silty clay loam; *B*, Trenton clay be worked out. We estimate that one man, properly equipped with sedimentation units and weighing bottles, could start about eight samples every day, and after obtaining the points indicated in the table finish each set of determinations in three or four days. If fewer points were sought, correspondingly more soils could be analyzed. This output of work would be from five to ten times that obtained by the method of the Bureau of Soils, for example.

TABLE 2

Rate of settling

DIAMETER	RADIUS	TIME TO FALL 10 CM.
mm.	*μ*	
0.25	125.0	1.9 seconds
0.1	50.0	12.0 seconds
0.05	25.0	48.0 seconds
0.01	5.0	20.0 minutes
0.005	2.5	80.0 minutes
0.002	1.0	8.33 hours
0.001	0.5	33.33 hours
0.0005	0.25	5.55 days

SUMMARY

1. A new method for the rapid and accurate mechanical analysis of soil is described which is applicable to a range of sizes from fine sand to colloidal material. The method may be used either for conventional routine work or for a detailed study of the size-frequency distribution of the particles. Only inexpensive apparatus such as may be found in any soils laboratory need be used.

2. Some experimental data are submitted showing that this method agrees closely with a reliable sedimentation method.

3. The method offers a quantitative means of studying flocculation phenomena. It should also yield valuable information concerning the colloidal properties of soil.

REFERENCES

(1) ALLEN, H. S. 1900 The motion of a sphere in a viscous liquid. *In* Phil. Mag., v. 5, no. 50, p. 323–338.

(2) ATTERBERG, A. 1912 Die mechanische Bodenanalyse und die Klassifikation der Mineralboden Schwedens. *In* Internat. Mitt. Bodenk., v. 2, p. 319.

(3) HALL, A. D. 1904 The mechanical analysis of soils and the composition of the fractions resulting therefrom. *In* Jour. Chem. Soc., v. 85, pt. 2, p. 950–963 (see especially page 959).

(4) JOSEPH, A. F., AND MARTIN, F. J. 1921 The determination of clay in heavy soil. *In* Jour. Agr. Sci., v. 11, pt. 3, p. 293–303.

(5) KNOTT, C. G. 1916 Mathematical note on the fall of small particles through liquid columns. *In* Proc. Roy. Soc. Edinb., v. 36, p. 237–239.

(6) Odén, S. 1916 The size of the particles in deep sea deposit. *In* Proc. Roy. Soc. of Edinb., v. 36, pp. 219–236.

(7) Odén, S. 1914–19 Bodenkundliche Forschungen an dem chemischen Universistäts-Laboratorium der Universität Uppsala, p. 301–342. Albert, Paul & Co., Berlin.

(8) Odén, S. 1922 Clays as disperse system. *In* Trans. Faraday Soc., v. 17, p. 13–34.

(9) Perrin, M. Jean 1910 Brownian movement and molecular reality, p. 34–40. Memoir (trans. by F. Soddy), Taylor and Francis, London.

(10) Robinson, G. W. 1915 Note on the effect of changes in the viscosity of water on the results of mechanical analysis conducted at varying temperatures. *In* Jour. Agr. Sci., v. 7, pt. 2, p. 142–143.

(11) Schloessing, T. 1903 On the mechanical analysis of soils. *In* Compt. Rend. Acad. Sci. (Paris), v. 136, p. 1608–1613; v. 137, p. 369–374, 394–402; *Abs. in* Exp. Sta. Rec., v. 15, p. 344.

(12) Wightman, E. P., and Sheppard, S. E. 1921 The size-frequency distribution of particles of silver halide in photographic emulsions. *In* Jour. Phys. Chem., v. 25, p. 561–594.

INDEX

Actinomycetes, microscopic method for demonstrating, 149–151
Actinomycetes scabies, effect of soil reaction on, 61–79
Adsorption—
  of salts by soil colloids, 1–7
  of Plant Food by Colloidal Silica (paper), R. C. Wiley and N. E. Gordon, 441–448
  of Plant Food by Soil Colloids, Influence of Hydrogen-Ion Concentration on the (paper), E. B. Starkey and N. E. Gordon, 449–457
Albrecht, W. A. (paper), Nitrate Accumulation under Straw Mulch, 299–305
Ali B. Wilsdon, B. H. and (paper), Nitrogen Fixation in Arid Climates, 127–133
Alkali soil, variability of, 177–189
Ammonification—
  in soil, effect of petroleum on, 465–477
Arrhenius O. (paper), A Possible Correlation between the Fertility of Rice Soils and their Titration Curves, 21–26
Arrhenius, O. (paper), The Potential Acidity of Soils, 223–232
Arsenic, Some Relations of, to Plant Growth: Part I (paper), John Stewart, 111–117
  solubility of lead arsenate—
  in salt solutions, 113–115
  in soil solution, 115–118
Arsenic, Some Relations of, to Plant Growth: Part 2 (paper), John Stewart, 119–126

Bacteria, nodule—
  movement of, in soil, 29–35
  effect of, on peas, 413–415
Baldwin, I. L. (paper), Modifications of the Soil Flora Induced by Applications of Crude Petroleum, 465–477
Blair, A. W., and Prince, A. L. (paper), Variation of Nitrate Nitrogen and pH Values of Soils from the Nitrogen Availability Plots, 9–20
Bouyoucos, G. J. (paper), Relation between Heat of Wetting, Moisture Equivalent and Unfree Water, 431–434
Bouyoucos, G. J., and McCool, M. M. (paper), A Study of the Causes of Frost Occurrence in Muck Soils, 383–389
Brown, P. E. (paper), Soil Experiment Fields and their Value, 369–376
Bryan, O. C.—
  Fred, E. B., and (paper), The Effect of Nodule Bacteria on the Yield and Nitrogen Content of Canning Peas, 413–415
  Fred, E. B., and (paper), The Formation of Nodules by Different Varieties of Soybeans, 417–420
Burgess, P. S. (paper), The Soil Solution, Extracted by Lipman's Direct-Pressure Method, Compared with 1:5 Water Extracts, 191–215

Calcium Compounds and other Substances, Effect of Certain, on the Yield and Calcium Content of Some Crops (paper), O. M. Shedd, 233–246
Colloids—
  adsorption of plant food by, 441–448, 449–457
  Soil, Influence of, on Availability of Salts (paper), N. E. Gordon and E. B. Starkey, 1–7
Conn, H. J. (paper), A Microscopic Method for Demonstratng Fungi and Actinomycetes in Soil, 149–151

Diffusion, the nature of, in acid phosphate manufactured by the Lipman process,

Erdman, L. W. (paper), The Sulfur Content of Rainwater, 363–367
Experimental results, value of, 369–376

Fertilizer—
  phosphatic limestone as a, 265–279
  sulfur as a, for cotton, 435–440
  value of field experiments on, 369–376
Flocculation, quantitative method for studying, 498
Frazier, W. C., and Fred, E. B. (paper), Movement of Legume Bacteria in Soil, 29–35

Fred, E. B.—
  and Bryan, O. C. (paper), The Effect of Nodule Bacteria on the Yield and Nitrogen Content of Canning Peas, 413–415
  and Bryan, O. C. (paper), The Formation of Nodules by Different Varieties of Soybeans, 417–420
  Frazier, W. C., and (paper), Movement of Legume Bacteria in Soil, 29–35
  Waksman, S. A.. and (paper), A Tentative Outline of the Plate Method for Determining the Number of Microorganisms in the Soil. 27–28
Frost Occurrence in Muck Soils, A Study of the Causes of (paper), G. J. Bouyoucos and M. M. McCool, 383–389
Fungi in the Soil, Growth of (paper), S. A. Waksman, 153–157
Fungi, microscopic method for demonstrating, 149–151

Gardner, W., Jennings, D. S., Thomas, M. D., and (paper), A New Method of Mechanical Analysis of Soil, 485–499
Gericke, W. F. (paper), Differences Effected in Protein Content of Grain by Applications of Nitrogen Made at Different Growing Periods of the Plants, 103–109
Gordon, N. E.—
  and Starkey, E. B. paper), Influence of Soil Colloids on Availability of Salts, 1–7
  Starkey, E. B. and (paper), Influence of Hydrogen-Ion Concentration on the Adsorpton of Plant Food by Soil Colloids, 449–457
  Wiley, R. C., and (paper). Adsorption of Plant Food by Colloidal Silica, 441–448
Greensand, sulfur oxidation in, 307–319

Helbronner, A., Rudolfs, W., and (paper), Oxidation of Zinc Sulfide by Microorganisms, 459–464
Hydrogen-ion concentration—
  importance of, in making acid phosphate by the Lipman process, 479–483
  of soil, effect of sulfur on, 247–263
  relation of,
    to adsorption by colloids, 1–7, 449–457
    to crop yields, 12
    to lime requirement, 11–12
  variation of, 9–20

Iron pyrites, oxidation of, by bacteria, 135–147
Irrigation—
  requirement of soils correlated with field moisture requirement and wilting point, 159–165
  Water, Some Ways of Increasing the Duty of, (paper), W. L. Powers, 377–382

Jennings, D. S, Thomas, M. D., and Gardner, W (paper), A New Method of Mechanical Analysis of Soils, 485–499
Joffe, J. S. (paper), Acid Phosphate Production by the Lipman Process: I. Effect of Moisture Content of Sulfur-floats-soil Mixtures on Sulfur Oxidation Activities, 479–483
Joffe, J. S., and McLean, H. C. (paper), A Note on Oxidation of Sulfur in Oregon Soils, 217–221

Kelley, W. P. (paper), Variability of Alkali Soil, 177–189

Latshaw, W. L., Swanson, C. O. (paper), Sulfur as an Important Fertility Element, 421–430
Legume Bacteria in Soil, Movement of (paper), W. C. Frazier and E. B. Fred, 29–35
Leidigh, A. H., Reynolds, E. B., and (paper), Sulfur as a Fertilizer for Cotton, 435–440
Lime—
  effect of, on soil flora, 321–346
  requirement—
    effect of soil acidity on, 15
    relation of, to crop yields, 12
    relation of, to pH values, 11–12
Limestone, phosphatic, as a fertilizer, 265–279
Lipman—
  direct-pressure method for extracting soil solution, 191–215
  process of acid phosphate manufacture, 479–483

McCool, M. M., Bouyoucos, G. J. and (paper), A Study of the Causes of Frost Occurrence in Muck Soils, 383–389
McLean, H. C., Joffe, J. S., and (paper), A Note on Oxidation of Sulfur in Oregon Soils, 217–221

Mechanical analysis of soil, new method of. 485–499
  Microbiological Analysis of Soil as an Index of Soil Fertility: I. The Mathematical Interpretation of Results Obtained from a Study of the Numbers of Microörganisms in the Soil (paper), S. A. Waksman, 81–101
    development of formula for probable error, 82–87
    relation of number of plates to error, 89–92
    relation of number of samples to error, 92–94
Microscopic Method for Demonstrating Fungi and Actinomycetes in Soil (paper) H. J. Conn, 149–151
Moisture as a factor in biological oxidation of sulfur, 479–483
Moisture-capacity of soils, 159–165
Mulch, nitrate accumulation under straw, 299–305

Nitrate Accumulation under Straw Mulch (paper), W. A. Albrecht, 299–305
Nitrates in soil—
  relation of, to crop yields, 11–12
  variation of, 9–20
Nitrification, effect of petroleum on, 465–477
Nitrogen—
  content of peas, effect of nodule bacteria on, 413–415
  effect of, on protein in grain, 103–109
  Fixation in Arid Climates (paper), Wilsdon, B. H., and Ali, B., 127–133
    relation to rainfall, 129
Nodule—
  Bacteria, The Effect of, on the Yield and Nitrogen Content of Canning Peas (paper), E. B. Fred and O. C. Bryan, 413–415
    formation, effect of sulfur on, 252–255
Nodules, The Formation of, by Different Varieties of Soybeans (paper), E. B. Fred and O. C. Bryan, 417–420

Oxygen, part played by, in biological oxidation of sulfur, 479–481

Petroleum, effect of, on soil flora, 465–477
Phosphate—
  composting rock, with sulfur, 37–59
  Acid, Production by the Lipman Process: I. Effect of Moisture Content of Sulfur-floats-soil Mixtures on Sulfur Oxidation (paper), J. S. Joffe, 479–481
  Rock, reactions involved in conversion of, 479–480
Phosphates, effect of pyrites on availability of, 135–147
Phosphatic Limestone, Calcined, as a Fertilizer (paper), E. Van Alstine, 265–279
Phosphorus, relation of silica to assimilation of, 347–362
Plant—
  composition, relation of silica and silicates to, 347–362
  food—
    adsorption of, by soil colloids, 441–448, 449–457
    calcium as a, 233–246
  growth—
    effect of
      arsenic on, 119–126
      fertilizer at different periods of, 103–109
      iron pyrites on, 143–145
      magnesium, 233–246
      silica and silicates on, 233–246
      sulfur and acid phosphate on, 248–258
      sulfur and rock phosphate on, 248–258
    of soybeans, effect of sulfur on, 247–263
    relation of silica and silicates to, 347–362
    relation of soil moisture to physiological salt balance, 391–411
Plate Method for—
  demonstrating soil fungi, 153–157
  Determining the Number of Microorganisms in the Soil, A tentative Outline of the (paper), S. A. Waksman and E. B. Fred, 27–28
Potassium, availability of, effect of sulfur in greensand on, 307–319
Powers, W. L. (paper)—
  Field Moisture Capacity and Wilting Point of Soils, 159–165
  Some Ways of Increasing the Duty of Irrigation Water, 377–382
Prince, A. L., Blair, A. W., and (paper), Variation of Nitrate Nitrogen and pH Values of Soils from the Nitrogen Availability Plots, 9–20
Probable error in microbiological soil analysis—
  development of formula for, 82–87

relation of number of plates, samples, and dilution to, 89–94
Protein—
  Content of Grain, Differences Effected in the, by Applications of Nitrogen Made at Different Growing Periods of the Plants (paper), W. F. Gericke, 103–109
  in grain, effect of nitrogen on, 103–109

Reynolds, E. B., and Leidigh, A. H. (paper), Sulfur as a Fertilizer for Cotton, 435–440
Rudolfs, W.—
  and Helbronner, A. (paper), Oxidation of Zinc Sulfide by Microörganisms, 459–464
  (paper), Composting Rock Phosphate with Sulfur in Slightly Calcareous, Soils; see Sulfur, Composting, etc. 37–59
  paper), Influence of Sulfur Oxidation upon Growth of Soy Beans and its Effect on Bacterial Flora of Soil, 247–263
  (paper), Oxidation of Iron Pyrites by Sulfur-Oxidizing Organisms, and their Use for making Mineral Phosphates Available; see Sulfur-Oxidizing Organisms, Oxidation of Iron Pyrites, etc., 135–147
  (paper), Sulfur Oxidation in Inoculated and Uninoculated Greensand Mixtures and its Relation to the Availability of Potassium, 307–319
Rost, C. O. (paper), Occurrence of Sulfides in Minnesota Peat Soils, 167–174
Rothamsted, The Human Element in the Work at (reprinted from Agricultural Gazette), 175–176

Schollenberger, C. J. (paper), Silica and Silicates in Relation to Plant Growth and Composition, 347–362
Shedd, O. M. (paper), Effect of Certain Calcium Compounds and other Substances on the Yield and Calcium Content of Some Crops, 233–246
Shive, J. W. (paper), The Relaton of Soil Moisture to Physiological Salt Balance for Plants, 391–411
Silica and Silicates in Relation to Plant Growth and Composition (paper), C. J. Schollenberger, 347–362
Smith, E. S., Stewart, J., and (paper), Some Relations of Arsenic to Plant Growth: Part 2, 119–126
Soil—
  colloids, adorption of plant food by, 441–448
  Experiment Fields and Their Value (paper), P. E. Brown, 369–376
  Fertility—
    Micobiologcal Analysis of Soil as an Index of, II. Methods of the Study of Numbers of Microörganisms in the Soil (paper), Waksman, S. A., 283–305
      limitations of methods, 285–287
      soil sampling, 287
      dilutions and plating, 287–288, 293–296
      incubation and counts, 288–289, 290–292
      influence of medium, 289–290, 292
      diluent, 296
    Microbiological Analysis of Soil as an Index of, III. Influence of Fertilization upon Numbers of Microorganisms in the Soil (paper), S. A. Waksman, 321–346
    relation of titration curves to, 21–26
    sulfur an element of, 42–430
  Flora—
    effect of sulfur on, 259–260
    Modifications of the, Induced by Applications of Crude Petroleum (paper), I. L. Baldwin, 465–477
  fungi, plate method for, 153–157
  heat conductivity of, effect on frost occurrence, 383–389
  microbiological analysis of, methods of, 283–305
  microörganisms, effect of fertilizers on, 321–346
  Moisture—
    equivalent, heat of wetting and unfree water, relation between, 431–434
    The Relation of, to Physiological Salt Balance for Plants, 391–411
  muck—
    frost occurrence in, 383–389
    sulfides in, 167–174
  peat, sulfides in, 167–174
  Reaction—
    effect on sulfides in peat and muck soils, 167–174

    - effect of sulfur on, 217–221
    - effect of sulfur on, 243–263
    - The Influence of, upon the Growth of Actinomycetes causing Potato Scab (paper), S. A. Waksman, 61–79
        - effect of acidity on various strains, 63–68
        - resistance of saprophytic species, 68–71
        - sulfur control, 73–76
- Solution—
    - Extracted by Lipman's Direct-Pressure Method, Compared with 1:5 Water Extracts, The (paper), P. S. Burgess, 191–215
- Variability of Alkali, (paper), W. P. Kelley, 177–189

Soils—
- A New Method of Mechanical Analysis of (paper), D. S. Jennings, M. D. Thomas, and W. Gardner, 485–497
- Field Moisture Capacity and Wilting Point of (paper), W. L. Powers, 159–165
- The Potential Acidity of (paper), O. Arrhenius, 223–232
    - buffer action of, and relation to fertility 223–232
    - potential acidity of and relation to fertility, 223–232

Starkey, E. B.—
- and Gordon, N. E. (paper), Influence of Hydrogen-Ion Concentration on the Adsorption of Plant Food by Soil Colloids, 449–457
- Gordon, N. E., and (paper), Influence of Soil Colloids on Availability of Salts, 1–7

Stewart, J.—
- (paper), Some Relations of Arsenic to Plant Growth: Part 1, 111–117
- and Smith, E. S. (paper), Some Relations of Arsenic to Plant Growth: Part 2, 119–126

Straw mulch, nitrate accumulation under 299–305

Sulfides in Minnesota Peat Soils, Occurrence of (paper), C. O. Rost, 167–174

Sulfur—
- as an Important Fertility Element (paper), C. O. Swanson and W. L. Latshaw, 421–430
- as a Fertilizer for Cotton (paper), E. B. Reynolds and A. H. Leidigh, 435–440
- Composting Rock Phosphate with, in Slightly Alkaline Calcareous Soils (paper), W. Rudolfs, 37–59
    - influence of—
        - light, 39–42
        - peptone, 39–42
        - temperature, 39–42
        - partial sterilization, 44–46
        - reducing quantity of sulfur, 49–50
        - initial reaction, 50–51
        - aeration, 51–56
    - stimulation by salts, 42–44
    - $(NH_4)_2SO_4$ as source of nitrogen, 47–49
- control of scab by, 73–76
- Content of Rainwater, The (paper), L. W. Erdman, 363–367
- effect of—
    - inoculation of, 217–221
    - on nodule formation, 252–255
    - on soil reaction, 73, 243–263
    - moisture on biological oxidation of, 479–483
- reactions involved in biological oxidation of, 479–481
- with acid phosphate, on plant growth, 248–258
- with rock phosphate, on plant growth, 248–258
- in Oregon Soils, A Note on Oxidation of, (paper), J. S. Joffe and H. C. McLean, 217–221
- method for determining, in soil, 421–430
- Oxidation (see Zinc Sulfide, Oxidation of, etc.), 459–467
    - in Inoculated and Uninoculated Greensand Mixtures and its Relation to the Availabiliy of Potassium (paper) W. Rudolfs, 307–319
    - Influence of, upon Growth of Soy Beans and its Effect on Bacterial Flora of Soil (paper), W. Rudolfs, 247–263

Sulfur-Oxidizing Organisms, Oxidation of Iron Pyrites by, and their Use for Making Mineral Phosphates Available (paper), W. Rudolfs, 135–147
- $(NH_4)_2SO_4$ as source of N, 140–141
- effect of aeration, 141–143
- effect of pyrites on plant growth, 143–145

Swanson, C. O., and Latshaw, W. L. (paper), Sulfur as an Important Fertility Element, 421–430

Titration Curves, A Possible Correlation between the Fertility of Rice Soils and their Titration Curves (paper), O. Arrhenius, 21–26

Thomas, M. D., and Gardner W., Jennings, D. S., (paper), A New Method of Mechanical Analysis of Soils, 485–499

Unfree water, discussion of, 191–215

Variability of alkali soil, 177–189

Variation of Nitrate Nitrogen and pH Values of Soils from the Nitrogen Availability Plots (paper), A. W. Blair and A. L. Prince, 9–20

Van Alstine, E. (paper), Calcined Phosphatic Limestone as a Fertilizer, 265–279

Waksman, S. A. (papers)—
- Microbiological Analysis of Soil as an Index of Soil Fertility: I. The Mathematical Interpretation of Results Obtained from a Study of the Numbers of Microörganisms in the Soil; see Microbiological Analysis, etc., 81–101
- Microbiological Analysis of Soil as an Index of Soil Fertility: II. Methods of the Study of Numbers of Microorganisms in the Soil, see Soil Fertility, Microbiological Analysis as an Index, etc., 283–305
- Microbiological Analysis of Soil as an Index of Soil Fertility: III. Influence of Fertilization upon Numbers of Microörganisms in the Soil, 321–346
- The Growth of Fungi in the Soil, 153–157
- The Influence of Soil Reaction upon the Growth of Actinomycetes Causing Potato Scab, see Soil Reaction, The Influence of, etc., 61–79
- and Fred, E. B. (paper), A Tentative Outline of the Plate Method for Determining the Number of Microorganisms in the Soil, 27–28

Water—
- extracts compared with solutions, direct-pressure, 191–215
- irrigation, increasing duty of, 377–382
- Unfree, Relation between Heat of Wetting, Moisture Equivalent and (paper), G. J. Bouyoucos, 431–434

Wiley, R. C., and Gordon, N. E. (paper), Adsorption of Plant Food by Colloidal Silica, 441–448

Wilsdon, B. H., and Ali, B. (paper), Nitrogen Fixation in Arid Climates, 127–133

Wilting point of soils, 159–165

Zinc Sulfide, Oxidation of, by Microörganisms (paper), W. Rudolfs and A. Helbronner, 459–464